Published in final edited form as:
J Steroid Biochem Mol Biol. 2011 October ; 127(1-2): 96–101. doi:10.1016/j.jsbmb.2010.12.005.

Molecular targets that link dioxin exposure to toxicity phenotypes☆

Wataru Yoshioka[a], **Richard E. Peterson**[b], and **Chiharu Tohyama**[a,*]

[a]Laboratory of Environmental Health Sciences, Center for Disease Biology and Integrative Medicine, Graduate School of Medicine, The University of Tokyo, 7-3-1 Bunkyo-ku, Tokyo 113-0033, Japan

[b]Molecular and Environmental Toxicology Center and Pharmaceutical Sciences Division, School of Pharmacy, University of Wisconsin, Madison, WI 53705, USA

Abstract

Many toxicology studies have elucidated health effects associated with exposure to various chemicals, but few have identified the molecular targets that cause specific endpoints of toxicity. Our understanding of the toxicity of dioxins, a group of chemicals capable of causing toxicity at environmentally relevant levels of exposure, is no exception. Dioxins are unique compared to most chemicals that we are exposed to in the environment because they activate a high affinity receptor, aryl hydrocarbon receptor (AhR), that was identified more than three decades ago. In recent years, several lines of experimental evidence have provided clues for opening the “black box” that contains the molecular mechanisms of dioxin action. These clues have emerged by toxicologists beginning to identify the molecular targets that link AhR signaling to tissue-specific toxicity phenotypes. Endpoints of dioxin toxicity for which downstream molecular targets have begun to be elucidated are observed in developmental or tissue regeneration processes, and include impaired prostate development and hydronephrosis in mouse fetuses and pups, reduced midbrain blood flow and jaw malformation in zebrafish embryos, and impaired fin regeneration in larval and adult zebrafish. Significant progress in identifying molecular targets for dioxin-induced hepatotoxicity in adult mice also has occurred. Misregulation of AhR downstream pathways, such as conversion of arachidonic acid to prostanoids via cyclooxygenase-2, and altered Wnt/β-catenin signaling downregulating Sox9, and signaling by receptors for inflammatory cytokines have been implicated in tissue-specific endpoints of dioxin toxicity. These findings may not only begin to clarify the molecular targets of dioxin action but shed light on new molecular events associated with development and disease.

Keywords

Aryl hydrocarbon receptor; Cyclooxygenase 2; Dioxin; Sox9; Toxicity

1. Introduction

Organisms, including humans have been exposed to thousands of environmental chemicals and adapt to such exposures or develop diseases that may last for a long evolutionary period. The majority of such chemicals act as xenobiotics to the organisms, and it is reasonable to

☆Article submitted for the special issue on Endocrine disruptors.

*Corresponding author. Tel.: +81 3 5841 1431; fax: +81 3 5841 1434. mtohyama@mail.ecc.u-tokyo.ac.jp (C. Tohyama). .

think that animals did not evolve and harbor receptors to respond to specific xenobiotics. Instead, organisms evolved detoxifying mechanisms, which have less stringent specificity to various xenobiotics, and the aryl hydrocarbon receptor (AhR) is no exception. The AhR is activated by various chemicals including a subset of polychlorinated dibenzo-*p*-dioxin (PCDD), dibenzofuran (PCDF) and biphenyl (PCB) congeners [1]. We will refer to these congeners as dioxin-like (DL) or simply as dioxins.

PCDDs and PCDFs consist of 75 and 135 congeners, respectively, but only those with chlorine substitutions at the 2,3,7, and 8 positions exhibit signs of 2,3,7,8-TCDD (abbreviated as TCDD hereafter) toxicity and are identified as DL-PCDDs and DL-PCDFs [1]. Accordingly, there are 7 DL-PCDD and 10 DL-PCDF congeners. The non-DL-PCDD and -PCDF congeners are metabolized more rapidly than the DL-2,3,7,8-congeners or do not bind the AHR. The DL-PCBs consist of 12 congeners. This includes 4 congeners that have no ortho chlorine substitutions on the biphenyl ring system. These are the most potent of the 12 DL-PCBs and 3,3′,4,4′,5-PCB (PCB126) is nearly as potent in causing toxicity as TCDD. The 8 mono-ortho chlorine substituted PCBs are very weak DL-PCBs with potencies 1/30,000th that of TCDD [2].

The DL-PCDDs, -PCDFs, and -PCBs are persistent, and ubiquitous environmental contaminants. They are found in air, water, soil, and sediment, and bioaccumulate in various animal species including humans worldwide. PCDDs and PCDFs are unintentional byproducts of combustion and various industrial activities. PCB mixtures were commercial products until banned and were widely used as heat resistant solvents, lubricants, and in fluorescent light ballasts. Among the DL-PCDDs, -PCDFs and -PCBs, TCDD is the most potent in causing toxicity and is the prototype to which biological and toxicological actions of all other DL-congeners are compared.

Epidemiological and clinical studies have shown that accidental and occupational exposure to DL-PCDDs, -PCDFs or -PCBs induce preclinical signs of abnormal health and various disease conditions. About 25 years after accidental exposure, the TCDD-exposed population in Seveso, Italy exhibited a skewed sex ratio at birth to girls[3]. Food poisoning by ingesting rice oil contaminated with DL-PCBs and PCDFs, known as *Yusho* and *Yu-cheng*, occurred in 1968 and 1979 in Japan and Taiwan, respectively [4,5]. The exposed populations had a variety of systemic signs and symptoms. Workers who were engaged in manufacturing a TCDD-contaminated herbicide were reported to have a higher risk of certain cancers [6]. It has been well-established that when laboratory animals are exposed to dioxins, they suffer a variety of toxicities, such as metabolic disorders and a wasting syndrome that eventually leads to death, reproductive toxicity and endocrine dysfunction, neurodevelopmental toxicity, immunosuppression, carcinogenicity and teratogenicity[7].

In this review, we provide a general overview of past research on the toxicology of TCDD with particular reference to the aryl hydrocarbon receptor (AhR). We then describe recent studies that have begun to provide insight into the molecular targets that may mediate tissue-specific endpoints of dioxin toxicity. These endpoints of toxicity relate mainly to developmental and regenerative processes that are disrupted by dioxin exposure. By the early 1970s occupational and accidental human exposures to TCDD and toxicity studies in laboratory animals demonstrated that TCDD was capable of causing some of the toxic effects described above, but the biological factors that established the link between dioxin exposure and these adverse effects were not understood. As a result, the underlying mechanisms of TCDD toxicity remained in a “black box”.

The first real clue to elucidating the mechanism was an attractive hypothesis for involvement of the AhR in the mid-1970s. A few research groups found that certain

polycyclic aromatic hydrocarbons, such as 3-methylcholanthrene, had the ability to induce aryl hydrocarbon hydroxylase activity, and that this ability segregated in mice as a single gene locus. In 1976, Alan Poland and his associates [8] reported the presence of a protein, AhR, that had high affinity for TCDD. Next, it was revealed how TCDD-bound AhR interacts with another transcription factor, the aryl hydrocarbon receptor nuclear translocator (ARNT), in the nucleus. The complex of these proteins was found to bind to the xenobiotic response element (XRE), also called the dioxin responsive element (DRE), in the promoter region of certain genes, such as CYP1A1, to activate transcription of these genes [9]. These target genes of AhR are related to physiological processes such as cell proliferation and drug metabolism, and to the manifestation of toxicities. The hypothesis that activation of AhR by TCDD leads to the abnormal transcription of genes, which in turn causes dioxin-like toxicities, is generally accepted, as the “genomic pathway” mechanism. Since the initial discovery of the genomic pathway of dioxin action, it has been reported that the AhR produces other actions through a “non-genomic pathway” [10] and is capable of cross-talk with other steroid hormone receptors [11]. These newer mechanisms of dioxin action involving the AhR are covered later in this review.

In late 1990s, three research groups [12-14] independently produced AhR-null mice by deleting different exons of the AhR gene. Although there seem to be some differences in the physiology of these animals, no overt dioxin toxicity could be found. That is, AhR-null mice did not show TCDD-inducible toxicities, such as thymic atrophy, immune suppression, wasting syndrome, cleft palate or hydronephrosis.

AhR conformation analysis revealed that differences in sensitivity to dioxin toxicities between species and strains can be generally attributable to polymorphism of the AhR. Although most of the amino acid changes in strains of mice occur within the transactivation domain, a point mutation in the ligand binding domain alters the affinity of AhR for dioxin, and susceptibility to dioxin toxicities. For example, C57BL/6 and DBA/2 mice are responsive and less responsive strains, respectively, and have different types of AhR that are characterized by their one order of magnitude difference in binding affinity for TCDD [15]. The dioxin dose used to produce toxic endpoints, such as lethality and abnormal lipid and carbohydrate metabolism, are one order of magnitude different between the two strains [16,17]. Furthermore, a required role for the AhR in the development of TCDD toxicity was shown by a study using the humanized AhR knock-in mouse [18]. In this study, AhR of the C57BL/6 strain mouse was replaced with an AhR of human origin, and TCDD-induced teratogenic endpoints in fetuses were compared between wild-type C57BL/6, DBA/2, and humanized C57BL/6. As expected, wild-type C57BL/6 mice were most responsive to TCDD and had the highest incidence of cleft palate and hydronephrosis compared to the DBA/2 strain. The humanized AhR knock-in mice on a C57BL/6 genetic background were the least sensitive to TCDD insult and had almost no indication of cleft palate and a low incidence of hydronephrosis.

It has been established that dioxin-bound AhR activates certain genes, such as CYP1A1, and induces various molecular and cellular responses. However, it was not clear how AhR target genes could eventually lead to specific endpoints of toxicity, although one could hypothesize that factors downstream of AhR signaling mediate toxic phenotypes in a tissue-specific manner. Despite continued research for more than 40 years, the molecules that link AhR activation by dioxin to specific endpoints of dioxin toxicity, have remained elusive.

We summarize below our current knowledge in this area by focusing on molecular targets that may mediate specific endpoints of dioxin developmental and regenerative toxicity. Since studies that have successfully identified actual target genes that are involved in

causing particular endpoints of toxicity are limited, additional studies of AhR downstream events that may lead to specific endpoints of dioxin toxicity are also included in this review.

2. Developmental and reproductive toxicities

2.1. AhR and Wnt/β-catenin pathway

In utero and lactational exposure to TCDD in rodents is a useful experimental model to study how TCDD affects the developmental stage of various organs, such as the prostate, mammary gland, and brain. Clarification of the molecular mechanism of TCDD action on prostate development is important for providing overarching information on prostate biology and possible clinical implications of AhR activation on prostate disease [19]. In the rodent, the prostate develops *in utero* from the urogenital sinus (UGS), to form three bilateral prostate lobes (ventral, dorsolateral, and anterior). TCDD has been shown to stimulate a paracrine signal, derived from AhR-mediated transcription in UGS mesenchyme (UGM), which inhibits prostatic bud formation in UGS epithelium (UGE) in the ventral lobe. Recently, Wnt5A localized in the UGM was shown to be involved in inhibition by TCDD of ventral prostate bud formation[20]. The addition of Wnt5A antibody rescued the prostatic budding from inhibition by TCDD. Thus, it is possible that abnormal upregulation of Wnt5A signaling occurred as a downstream event of TCDD-activated AhR signaling in the UGM [19].

Disruption of Wnt signaling by TCDD also has been found to induce toxicities in zebrafish (*Daniorerio*). Since the AhR signal transduction pathway in zebrafish is very similar to that of mammals, with an exception that zebrafish possess three AHRs, AhR1a AhR1b and AhR2 [21]. Zebrafish have become a useful model to delineate molecular mechanisms of TCDD toxicity. In zebrafish, AhR2 only is activated by TCDD to induce CYP1A and CYP1B. In developing zebrafish larvae, TCDD-induced adverse effects, including pericardial edema, slowed peripheral blood flow, craniofacial malformation and defects in erythropoiesis, could not be rescued by suppression of CYP1A and 1B using antisense morpholino oligonucleotides [22,23]. Thus, it is not likely that these particular AhR target genes, CYP1A and CYP1B are responsible for these endpoints of TCDD developmental toxicity in zebrafish. In mice there seems to be contradictory results regarding the involvement of CYP1A1 in specific endpoints of TCDD toxicity. Even though a high dose of TCDD (200 μg/kg) was not lethal in CYP1A1-null mice, these mice displayed many other hallmark signs of TCDD toxicity, demonstrating that induction of CYP1A1 is not required for most endpoints of dioxin toxicity [24]. On the other hand, TCDD exposure (180 ng/kg for 35 days) in adult mice produces reactive oxygen species via CYP1A1 that resulted in a continued elevation of blood pressure[25]. Taken together with many other relevant studies, it is concluded that the AhR is important for the manifestation of TCDD toxicity in a variety of vertebrate animals.

To identify the downstream events of AhR2 activation that are essential for dioxin toxicity, Tanguay and coworkers [26] employed the zebrafish caudal fin regeneration model where AhR2 activation by TCDD blocks regeneration in both adult and larval stages. Using genomic analysis for zebrafish at these two stages of development, a common alteration in gene expression was discovered. More specifically, R-Spondin1, a secreted protein capable of promoting Wnt/β-catenin signaling, and Sox9b had the highest and lowest expression, respectively [27,28]. Repression of R-Spondin1 by its antisense morpholino oligonucleotide protected against the TCDD-induced suppression of fin regeneration. When the Wnt coreceptor LRP6 was suppressed by its antisense morpholino oligonucleotide, inhibition of fin regeneration by TCDD also was blocked, demonstrating that inappropriate upregulation of R-Spondin/LRP6 is necessary for TCDD to inhibit fin regeneration. To our knowledge, this discovery is one of few investigations that actually identified the molecular target for a

specific endpoint of TCDD toxicity. An emerging question is how the upregulated R-Spondin1 that results in activation of Wnt/LRP6 leads to downregulation of Sox9b and eventually impairs fin regeneration.

Xiong et al. [29] reported that zebrafish larvae exposed to TCDD had impairment of lower jaw formation, and that Sox9b was significantly downregulated. Such impairment of lower jaw formation by TCDD was rescued by injection of Sox9b mRNA in the zebrafish embryo. Furthermore, morpholino knock-down of Sox9b in control zebrafish embryos caused a lower jaw malformation that was essentially identical to that caused by TCDD. Understanding how altered AhR, R-Spondin, Wnt/β-catenin, and Sox9 signaling elicits specific endpoints of TCDD toxicity merits further investigation (Fig. 1, left).

2.2. AhR, cyclooxygenase, and prostanoid pathway

Exposure to TCDD *in utero* or via lactation induces hydronephrosis in rodent fetuses and pups in an AhR-dependent manner [30]. It has also been reported that congenital obstructive nephropathy is the most frequent cause of renal failure in infants and children[31]. According to clinical studies, fetal hydronephrosis is found in approximately 1 in 100 births, with at least 20% being clinically significant [32]. Hydronephrosis is defined as a dilation of the renal pelvis and calyces proximal to the point of ureter obstruction[33]. Most of the obstruction has been found in the narrow junction between the renal pelvis and the ureter. In some cases, abnormal peristaltic movement of the ureter is also known to cause functional hydronephrosis.

Compiling data from *in vivo* and *in vitro* studies, a working hypothesis for the mechanism of TCDD-induced hydronephrosis was formulated (Fig. 1). The background information which led to this hypothesis is as follows. First, mice lacking the Na^+ and K^+ transporters, NKCC2 and ROMK, are thought to be a disease model for Bartter's syndrome and develop polyuria and severe hydronephrosis [34-36]. Second, PGE_2 and its receptors are involved in the regulation of NKCC2 and ROMK expression *in vitro* [37] and in PGE_2 receptor-null mouse studies [38]. Third, cyclooxygenase-2 (COX-2), an inducible rate-limiting enzyme for the production of prostanoids including PGE_2, is induced by TCDD in hepatocytes *in vitro* [39]. Fourth, expression of COX-2 is induced by multiple mechanisms including upregulation of inflammatory cytokines [40] and by the Src pathway [41].

In this mouse model, pups were exposed to TCDD via lactation from postnatal day (PND) 1, and nearly all pups developed hydronephrosis by weaning. Although COX-2 was induced in cultured cells [39], this study was probably the first to show that TCDD administration induced COX-2 mRNA and protein *in vivo*[42]. Recent studies on the human COX-2 promoter demonstrated that it has binding sites for transcription factors, and that CREB and AP-1 proteins, C/EBP protein, and NF-κB can presumably bind to these sites and activate COX-2 expression [43]. Thus, COX-2 can be induced by multiple mechanisms.

Another possible mechanism for COX-2 induction by TCDD involves a non-genomic pathway that is not accompanied by AhR translocation to the nucleus [44]. According to this hypothesis, COX-2 is induced by arachidonic acid release via the elevation of cytosolic phospholipase A2 (cPLA2) activity. When the COX-2 selective inhibitor, indomethacin N-octyl amide, was administered to pups from PND 1 to weaning, the altered expression of COX-2 activity, Na^+ and K^+ transporter genes, and inflammatory genes was reversed, and eventually, the onset of hydronephrosis was completely prevented [42]. The required role for increased COX-2 activity in TCDD-induced hydronephrosis was further confirmed by lithium treatment, which increases COX-2 activity to elicit hydronephrosis when administered to control mouse neonates from PND1 to weaning [45]. Thus, COX-2 is a necessary factor for inducing hydronephrosis in the developing rodent kidney.

Teraoka and associates also found that COX-2 is a target molecule of mesencephalic circulation failure caused by TCDD in zebrafish embryos [46]. TCDD reduced blood flow in the mesencephalic vein, and this circulation failure was prevented by treatment with two selective COX-2 inhibitors, NS-398 and SC-236. Also the mesencephalic circulation failure caused by TCDD was rescued by knocking down COX-2 activity. This TCDD-induced inhibitory effect on regional brain blood flow in zebrafish larvae was also blocked by selective antagonists of the thromboxane receptor (TP). Furthermore, exposure of zebrafish embryos to a TP agonist reduced mesencephalic vein blood flow that was blocked by a TP antagonist. Suppression of thromboxane (TX) A synthase 1 activity by antisense morpholino oligonucleotides protected zebrafish embryos from TCDD-induced mesencephalic circulation failure. It is concluded that the COX-2, TXA synthase, and TP axis plays a pivotal role in the reduced circulation in the developing midbrain of TCDD-exposed zebrafish larvae.

3. AhR and non-genomic pathway

Matsumura and associates proposed that TCDD-induced toxicity via a non-genomic pathway [47]. They performed an *in vitro* study using MCF10A cells to identify TCDD-induced inflammatory markers, such as vascular endothelial growth factor, and plasminogen activator inhibitor-2 mRNAs which resulted in four major findings. First, TCDD effectively induced these inflammation markers via calcium ions, cPLA2 activation, and Src kinase activation. Such findings were confirmed by the use of specific inhibitors for Src kinase and cPLA2, as well as calcium-signaling blockers. Second, TCDD activated Src kinases within 30 min, which was transferred from the cytoplasm to the plasma membrane. This TCDD-inducible Src activation was suppressed by a competitive inhibitor for cPLA2, suggesting that suppression of c-Src activation is an event down-stream of cPLA2 signaling. In addition, knockdown or inhibition of cPLA2 abolished the ability of TCDD to induce COX-2, suggesting that COX-2 induction is located downstream of cPLA2 signaling. Third, activation of cPLA2 and Src genes occurred within 15-30 min after TCDD addition to the culture medium, a time when induction of CYP1A1 could not be detected. Also in the above study on TCDD-induced hydronephrosis [42], lactational exposure to TCDD induced various inflammatory cytokines and chemokines in pups. Monocyte chemoattractant protein-1 was elevated as early as PND 3 and tumor necrosis factor-α (TNF-α) and IL-1β were upregulated as early as PND 5. Thus, the more rapid COX-2 induction by TCDD may be triggered by arachidonic acid signaling; not by inflammatory signaling. Fourth, knockdown of ARNT showed that TCDD-induced upregulation of the above mentioned inflammatory markers does not require ARNT, whereas the presence of ARNT is required for the induction of CYP1A1 mRNA.

TCDD exposure causes cardiovascular abnormalities in zebrafish embryos [48] and recently it has been shown that *medaka* embryos exposed to TCDD have inflammation and the altered prostanoid signaling, including dose-dependent upregulation of COX-2, which results in pericardial edema [49]. In the *medaka* model, arachidonic acid treatment also induced pericardial edema, but did not upregulate CYP1A1, indicating that pericardial edema can be induced by TCDD, independent of AhR/ARNT interaction with the XRE. The *medaka* results also support the presence of a non-genomic action of TCDD for inducing cellular responses which may play a role in certain cardiovascular endpoints of dioxin toxicity.

4. AhR and its interaction with steroid hormone receptors and other proteins

AhR binds heat shock protein 90, AIP (AhR-interacting protein also known as XAP2 and ARA9), and p23 in the cytoplasm. Upon binding TCDD, the ligand activated-AhR translocates into the nucleus, and heterodimerizes with ARNT. The liganded AhR/ARNT complex then binds to the XRE in the promoter region of AhR target genes and activates transcription of some genes cooperatively with transactivating factors, such as SP1 and p-TEFb. Mice having an AhR that cannot bind the XRE do not develop signs of TCDD toxicity, such as hepatomegaly, thymic involution and cleft palate [50], demonstrating an imperative role for the XRE in the development of these endpoints of TCDD toxicity. The majority of TCDD-induced hepatotoxicity also is not observed in mice lacking AIP, showing that AIP plays an essential role in causing this endpoint of toxicity [51]. AIP-null mice have unique characteristics, such as having 60% less AhR expression and differential expression among AhR-dependent CYP genes such as upregulated expression of CYP1A1 and 1A2 and downregulated expression of CYP1B1. This underlines the importance of the interaction of AhR and AIP for the expression of certain AhR target genes [51]. The same research group previously reported that a triple-knockout mouse model that lacks receptors for TNF-α and TNF-β, respectively, and a receptor for IL1-α and IL1-β had significant attenuation of TCDD-induced liver inflammation and hepatocellular damage [52]. The involvement of AIP and IL-1-like cytokines, and their interactions with AhR pathway in eliciting TCDD-induced inflammatory effects in the liver is an emerging area of AhR biology.

Ohtake and associates [53] reported that the ligand-bound-AhR/ARNT complex has the ability to activate estrogen receptor target genes, such as c-fos and VEGF by hijacking estrogen receptors and recruiting both estrogen receptors and p300, a coactivator, to the estrogen response element in the promoter region of estrogen receptor target genes. Importantly, TCDD has uterotropic effects in ovariectomized mice. Similarly, the ligand-bound AhR/ARNT complex can activate the androgen receptor. These results suggest that TCDD exposure is capable of modulating certain steroid hormone actions which might be involved in TCDD toxicity.

In contrast to the induction of gene expression, the TCDD-bound AhR/ARNT complex was reported to suppress, albeit not entirely, gene expression of c-fos in a human mammary gland tumor cell line, MCF-7, in an XRE-dependent manner. Since an estrogen responsive GC-rich site containing an SP-1 binding element in the promoter region of c-fos overlaps XRE in the same region, binding of the ligand-bound AhR-ARNT complex to the XRE was suggested to quench estrogen-dependent activation of estrogen receptor target genes [54]. More recently Ohtake and associates [55] showed that the ligand-bound AhR/ARNT complex acts as a constituent of E3 ubiquitin ligase to stimulate degradation of estrogen and androgen receptors, suppressing sex steroid hormone signaling. Thus, significant cross-talk occurs between AhR signaling and estrogen and androgen receptor signaling. This cross-talk has the potential to modulate cellular responses and biological endpoints induced not only by DL-PCDDs, DL-PCDFs and DL-PCBs but also by estrogens, androgens and other chemicals that modulate activity of these signaling pathways.

5. Future research directions

The molecular mechanism of dioxin toxicity involving AhR/ARNT signaling is based on a strong foundation of experimental evidence of AhR/ARNT-dependent genomic alterations. The non-genomic mechanism based on *in vitro* experimental evidence is new, has less firm scientific support but is emerging as another plausible model of TCDD action that may

occur *in vivo*. Although it is known that most endpoints of dioxin toxicity are induced via activation of AhR/ARNT signaling, the genomic pathway; more recent evidence is accumulating to show that other transcription factors cross-talk with AhR/ARNT signaling and may play a role in the modulation of various endpoints of toxicity caused by TCDD.

Recently, we found that TCDD treatment of adult mice resulted in downregulation of miR-101a, followed by elevation of it is target gene, COX-2, in the liver, leading to inflammatory liver injury [56]. Determining whether downregulation of miR-101a by TCDD occurs as an early event, before the onset of hydronephrosis in the rodent fetus and mesencephalic circulation failure in the zebrafish embryo, warrants further investigation.

In summary, determining the physiological and pathological significance of the various factors mentioned above in causing specific endpoints of dioxin toxicity will deepen our understanding of the human health risk posed by exposure to dioxins in the environment. After four decades of investigating the biology of AhR signaling and identifying endpoints of dioxin toxicity, we have now reached the stage of “molecular target toxicology”. That is, identifying molecular targets of TCDD action that trigger specific endpoints of toxicity. Based on the recent progress we have made towards this goal, as highlighted in this review, our knowledge of AhR biology and dioxin toxicology is sure to increase significantly in the future.

Acknowledgments

This work was supported in part by a grant-in-aid from JSPS (No. 21390186 to C.T. and 21710066 to W.Y.) and by the Environment Research and Technology Development Fund (C-0906 to C.T.) of the Ministry of the Environment, Japan, and by an NIEHS grant (ES01332 to R.E.P.).

References

[1]. World Health Organization. Consultation on assessment of the health risk of dioxins; re-evaluation of the tolerable daily intake (TDI): executive summary. Food Addit. Contam. 2000; 17:223–240.

[2]. Van den Berg M, Birnbaum LS, Denison M, De Vito M, Farland W, Feeley M, Fiedler H, Hakansson H, Hanberg A, Haws L, Rose M, Safe S, Schrenk D, Tohyama C, Tritscher A, Tuomisto J, Tysklind M, Walker N, Peterson RE. The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicol. Sci. 2006; 93:223–241. [PubMed: 16829543]

[3]. Mocarelli P, Gerthoux PM, Ferrari E, Patterson DG Jr. Kieszak SM, Brambilla P, Vincoli N, Signorini S, Tramacere P, Carreri V, Sampson EJ, Turner WE, Needham LL. Paternal concentrations of dioxin and sex ratio of offspring. Lancet. 2000; 355:1858–1863. [PubMed: 10866441]

[4]. Kuratsune, M.; Yoshimura, H.; Hori, Y.; Okumura, M.; Masuda, Y., editors. Yusho: A Human Disaster Caused by PCBs and Related Compounds. Kyushu University Press; Fukuoka: 1996.

[5]. Guo, YL.; Yu, ML.; Hsu, CC. The Yucheng rice oil poisoning incident. In: Shecter, A.; Gasiewicz, TA., editors. Dioxins and Health. John Wiley & Sons; Hoboken, NJ, USA: 2003. p. 893-919.

[6]. International Agency for Research on Cancer. Polychlorinated Dibenzo-paradioxins and Polychlorinated Dibenzofurans. IARC; Lyon, France: 1997.

[7]. Schecter, A.; Gasiewicz, TA., editors. Dioxin and Health. John Wiley & Sons; Hoboken: 2003.

[8]. Poland A, Glover E, Kende AS. Stereospecific, high affinity binding of 2,3,7,8-tetrachlorodibenzo-p-dioxin by hepatic cytosol. Evidence that the binding species is receptor for induction of aryl hydrocarbon hydroxylase. J. Biol. Chem. 1976; 251:4936–4946. [PubMed: 956169]

[9]. Mimura J, Fujii-Kuriyama Y. Functional role of AhR in the expression of toxic effects by TCDD. Biochim. Biophys. Acta. 2003; 1619:263–268. [PubMed: 12573486]

[10]. Matsumura F. The significance of the nongenomic pathway in mediating inflammatory signaling of the dioxin-activated Ah receptor to cause toxic effects. Biochem. Pharmacol. 2009; 77:608–626. [PubMed: 18996358]

[11]. Ohtake F, Baba A, Fujii-Kuriyama Y, Kato S. Intrinsic AhR function underlies cross-talk of dioxins with sex hormone signalings. Biochem. Biophys. Res. Commun. 2008; 370:541–546. [PubMed: 18358233]

[12]. Schmidt JV, Su GH, Reddy JK, Simon MC, Bradfield CA. Characterization of a murine Ahr null allele: involvement of the Ah receptor in hepatic growth and development. Proc. Natl. Acad. Sci. U.S.A. 1996; 93:6731–6736. [PubMed: 8692887]

[13]. Mimura J, Yamashita K, Nakamura K, Morita M, Takagi TN, Nakao K, Ema M, Sogawa K, Yasuda M, Katsuki M, Fujii-Kuriyama Y. Loss of teratogenic response to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in mice lacking the Ah (dioxin) receptor. Genes Cells. 1997; 2:645–654. [PubMed: 9427285]

[14]. Gonzalez FJ, Fernandez-Salguero P. The aryl hydrocarbon receptor: studies using the AHR-null mice. Drug Metab. Dispos. 1998; 26:1194–1198. [PubMed: 9860927]

[15]. Okey AB, Franc MA, Moffat ID, Tijet N, Boutros PC, Korkalainen M, Tuomisto J, Pohjanvirta R. Toxicological implications of polymorphisms in receptors for xenobiotic chemicals: the case of the aryl hydrocarbon receptor. Toxicol. Appl. Pharmacol. 2005; 207:43–51. [PubMed: 15993909]

[16]. Chapman DE, Schiller CM. Dose-related effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in C57BL/6J and DBA/2J mice. Toxicol. Appl. Pharmacol. 1985; 78:147–157. [PubMed: 4035666]

[17]. Okey AB, Vella LM, Harper PA. Detection and characterization of a low affinity form of cytosolic Ah receptor in livers of mice nonresponsive to induction of cytochrome P1-450 by 3-methylcholanthrene. Mol. Pharmacol. 1989; 35:823–830. [PubMed: 2543914]

[18]. Moriguchi T, Motohashi H, Hosoya T, Nakajima O, Takahashi S, Ohsako S, Aoki Y, Nishimura N, Tohyama C, Fujii-Kuriyama Y, Yamamoto M. Distinct response to dioxin in an arylhydrocarbon receptor (AHR)-humanized mouse. Proc. Natl. Acad. Sci. U.S.A. 2003; 100:5652–5657. [PubMed: 12730383]

[19]. Vezina CM, Lin TM, Peterson RE. AHR signaling in prostate growth, morphogenesis, and disease. Biochem. Pharmacol. 2009; 77:566–576. [PubMed: 18977204]

[20]. Allgeier SH, Lin TM, Vezina CM, Moore RW, Fritz WA, Chiu SY, Zhang C, Peterson RE. WNT5A selectively inhibits mouse ventral prostate development. Dev. Biol. 2008; 324:10–17. [PubMed: 18804104]

[21]. Hahn ME, Karchner SI, Evans BR, Franks DG, Merson RR, Lapseritis JM. Unexpected diversity of aryl hydrocarbon receptors in non-mammalian vertebrates: insights from comparative genomics. J. Exp. Zool. A Comp. Exp. Biol. 2006; 305:693–706. [PubMed: 16902966]

[22]. Carney SA, Peterson RE, Heideman W. 2,3,7,8-Tetrachlorodibenzo-p-dioxin activation of the aryl hydrocarbon receptor/aryl hydrocarbon receptor nuclear translocator pathway causes developmental toxicity through a CYP1A-independent mechanism in zebrafish. Mol. Pharmacol. 2004; 66:512–521. [PubMed: 15322242]

[23]. Yin HC, Tseng HP, Chung HY, Ko CY, Tzou WS, Buhler DR, Hu CH. Influence of TCDD on zebrafish CYP1B1 transcription during development. Toxicol. Sci. 2008; 103:158–168. [PubMed: 18308702]

[24]. Uno S, Dalton TP, Sinclair PR, Gorman N, Wang B, Smith AG, Miller ML, Shertzer HG, Nebert DW. Cyp1a1(-/-) male mice: protection against high-dose TCDD-induced lethality and wasting syndrome, and resistance to intrahepatocyte lipid accumulation and uroporphyria. Toxicol. Appl. Pharmacol. 2004; 196:410–421. [PubMed: 15094312]

[25]. Kopf PG, Scott JA, Agbor LN, Boberg JR, Elased KM, Huwe JK, Walker MK. Cytochrome P4501A1 is required for vascular dysfunction and hypertension induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol. Sci. 2010

[26]. Mathew LK, Simonich MT, Tanguay RL. AHR-dependent misregulation of Wnt signaling disrupts tissue regeneration. Biochem. Pharmacol. 2009; 77:498–507. [PubMed: 18938144]

[27]. Andreasen EA, Mathew LK, Tanguay RL. Regenerative growth is impacted by TCDD: gene expression analysis reveals extracellular matrix modulation. Toxicol. Sci. 2006; 92:254–269. [PubMed: 16443690]

[28]. Mathew LK, Sengupta SS, Ladu J, Andreasen EA, Tanguay RL. Crosstalk between AHR and Wnt signaling through R-Spondin1 impairs tissue regeneration in zebrafish. FASEB J. 2008; 22:3087–3096. [PubMed: 18495758]

[29]. Xiong KM, Peterson RE, Heideman W. Aryl hydrocarbon receptor-mediated down-regulation of sox9b causes jaw malformation in zebrafish embryos. Mol. Pharmacol. 2008; 74:1544–1553. [PubMed: 18784347]

[30]. Theobald, HM.; Kimmel, GL.; Peterson, RE. Developmental and reproductive toxicity of dioxins and related chemicals. In: Schecter, A.; Gasiewicz, TA., editors. Dioxin and Health. John Wiley & Sons; Hoboken, NJ, USA: 2003. p. 329-432.

[31]. Chevalier RL, Thornhill BA, Forbes MS, Kiley SC. Mechanisms of renal injury and progression of renal disease in congenital obstructive nephropathy, Pediatr. Nephrol. 2010; 25:687–697.

[32]. Belarmino JM, Kogan BA. Management of neonatal hydronephrosis. Early Hum. Dev. 2006; 82:9–14. [PubMed: 16427220]

[33]. Zeidel, JL.; Pirtskhalaishvili, G. Urinary tract obstruction. In: Brenner, BM., editor. Brenner and Rector's The Kidney. Saunders; Philadelphia: 2004. p. 1867-1894.

[34]. Takahashi N, Chernavvsky DR, Gomez RA, Igarashi P, Gitelman HJ, Smithies O. Uncompensated polyuria in a mouse model of Bartter's syndrome. Proc. Natl. Acad. Sci. U.S.A. 2000; 97:5434–5439. [PubMed: 10779555]

[35]. Lorenz JN, Baird NR, Judd LM, Noonan WT, Andringa A, Doetschman T, Manning PA, Liu LH, Miller ML, Shull GE. Impaired renal NaCl absorption in mice lacking the ROMK potassium channel, a model for type II Bartter's syndrome. J. Biol. Chem. 2002; 277:37871–37880. [PubMed: 12122007]

[36]. Lu M, Wang T, Yan Q, Yang X, Dong K, Knepper MA, Wang W, Giebisch G, Shull GE, Hebert SC. Absence of small conductance K^+ channel (SK) activity in apical membranes of thick ascending limb and cortical collecting duct in ROMK (Bartter's) knockout mice. J. Biol. Chem. 2002; 277:37881–37887. [PubMed: 12130653]

[37]. Kaji DM, Chase HS Jr. Eng JP, Diaz J. Prostaglandin E2 inhibits Na-K-2Cl cotransport in medullary thick ascending limb cells. Am. J. Physiol. 1996; 271:C354–361. [PubMed: 8760065]

[38]. Nusing RM, Treude A, Weissenberger C, Jensen B, Bek M, Wagner C, Narumiya S, Seyberth HW. Dominant role of prostaglandin E2 EP4 receptor in furosemide-induced salt-losing tubulopathy: a model for hyperprostaglandin E syndrome/antenatal Bartter syndrome. J. Am. Soc. Nephrol. 2005; 16:2354–2362. [PubMed: 15976003]

[39]. Puga A, Hoffer A, Zhou S, Bohm JM, Leikauf GD, Shertzer HG. Sustained increase in intracellular free calcium and activation of cyclooxygenase-2 expression in mouse hepatoma cells treated with dioxin. Biochem. Pharmacol. 1997; 54:1287–1296. [PubMed: 9393671]

[40]. Yang T. Regulation of cyclooxygenase-2 in renal medulla. Acta Physiol. Scand. 2003; 177:417–421. [PubMed: 12648158]

[41]. Matsumura F. On the significance of the role of cellular stress response reactions in the toxic actions of dioxin. Biochem. Pharmacol. 2003; 66:527–540. [PubMed: 12906918]

[42]. Nishimura N, Matsumura F, Vogel CF, Nishimura H, Yonemoto J, Yoshioka W, Tohyama C. Critical role of cyclooxygenase-2 activation in pathogenesis of hydronephrosis caused by lactational exposure of mice to dioxin. Toxicol. Appl. Pharmacol. 2008; 231:374–383. [PubMed: 18606429]

[43]. Nie M, Pang L, Inoue H, Knox AJ. Transcriptional regulation of cyclooxygenase 2 by bradykinin and interleukin-1beta in human airway smooth muscle cells: involvement of different promoter elements transcription factors, and histone h4 acetylation. Mol. Cell Biol. 2003; 23:9233–9244. [PubMed: 14645533]

[44]. Dong B, Nishimura N, Vogel CF, Tohyama C, Matsumura F. TCDD-induced cyclooxygenase-2 expression is mediated by the nongenomic pathway in mouse MMDD1 macula densa cells and kidneys. Biochem. Pharmacol. 2010; 79:487–497. [PubMed: 19782052]

[45]. Yoshioka W, Akagi T, Nishimura N, Shimizu H, Watanabe C, Tohyama C. Severe toxicity and cyclooxygenase (COX)-2 mRNA increase by lithium in the neonatal mouse kidney. J. Toxicol. Sci. 2009; 34:519–525. [PubMed: 19797860]

[46]. Teraoka H, Kubota A, Dong W, Kawai Y, Yamazaki K, Mori C, Harada Y, Peterson RE, Hiraga T. Role of the cyclooxygenase 2-thromboxane pathway in 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced decrease in mesencephalic vein blood flow in the zebrafish embryo. Toxicol. Appl. Pharmacol. 2009; 234:33–40. [PubMed: 18952116]

[47]. Park S, Dong B, Matsumura F. Rapid activation of c-Src kinase by dioxin is mediated by the Cdc37-HSP90 complex as part of Ah receptor signaling in MCF10A cells. Biochemistry. 2007; 46:899–908. [PubMed: 17223712]

[48]. Carney SA, Prasch AL, Heideman W, Peterson RE. Understanding dioxin developmental toxicity using the zebrafish model. Birth Defects Res. A Clin. Mol. Teratol. 2006; 76:7–18. [PubMed: 16333842]

[49]. Dong W, Matsumura F, Kullman SW. TCDD induced pericardial edema and relative COX-2 expression in *medaka* (Oryzias latipes) embryos. Toxicol. Sci. 2010

[50]. Bunger MK, Glover E, Moran SM, Walisser JA, Lahvis GP, Hsu EL, Bradfield CA. Abnormal liver development and resistance to 2,3,7,8-tetrachlorodibenzo-p-dioxin toxicity in mice carrying a mutation in the DNA-binding domain of the aryl hydrocarbon receptor. Toxicol. Sci. 2008; 106:83–92. [PubMed: 18660548]

[51]. Nukaya M, Lin BC, Glover E, Moran SM, Kennedy GD, Bradfield CA. The aryl hydrocarbon receptor-interacting protein (AIP) is required for dioxin-induced hepatotoxicity but not for the induction of the Cyp1a1 and Cyp1a2 genes. J. Biol. Chem. 2010; 285:35599–35605. [PubMed: 20829355]

[52]. Pande K, Moran SM, Bradfield CA. Aspects of dioxin toxicity are mediated by interleukin 1-like cytokines. Mol. Pharmacol. 2005; 67:1393–1398. [PubMed: 15722456]

[53]. Ohtake F, Baba A, Takada I, Okada M, Iwasaki K, Miki H, Takahashi S, Kouzmenko A, Nohara K, Chiba T, Fujii-Kuriyama Y, Kato S. Dioxin receptor is a ligand-dependent E3 ubiquitin ligase. Nature. 2007; 446:562–566. [PubMed: 17392787]

[54]. Duan R, Porter W, Samudio I, Vyhlidal C, Kladde M, Safe S. Transcriptional activation of c-fos protooncogene by 17 beta-estradiol: mechanism of aryl hydrocarbon receptor-mediated inhibition. Mol. Endocrinol. 1999; 13:1511–1521. [PubMed: 10478842]

[55]. Ohtake F, Fujii-Kuriyama Y, Kato S. AhR acts as an E3 ubiquitin ligase to modulate steroid receptor functions. Biochem. Pharmacol. 2009; 77:474–484. [PubMed: 18838062]

[56]. Yoshioka, W.; Higashiyama, W.; Tohyama, C. Reduction in Mir-101a Expression Level and Associated Changes in TCDD-Exposed Mouse Liver; 49th Annual Meeting and ToxExpo; Oxford University Press, Salt Lake City Utah, USA. 2010; p. 365

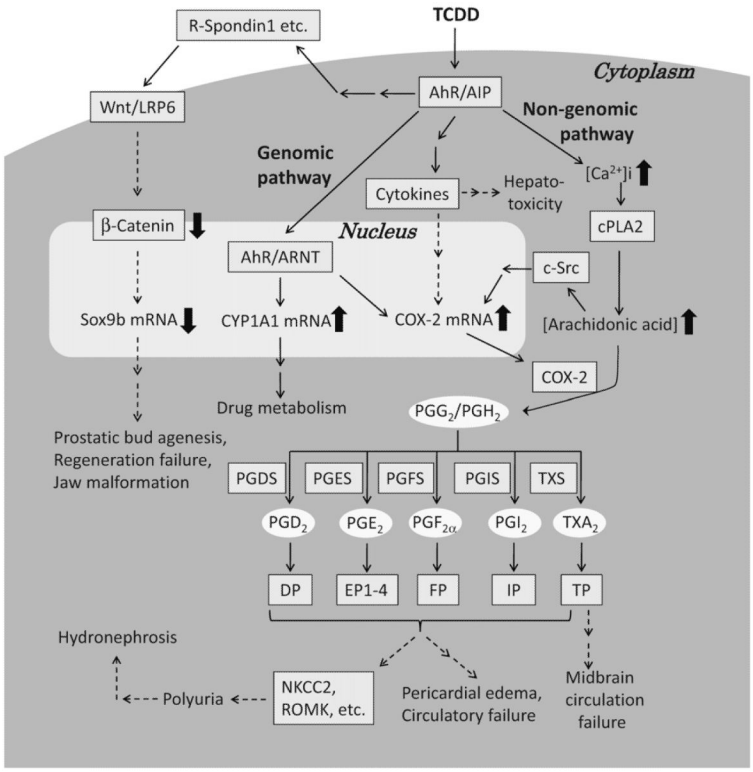

Fig. 1.

Proposed scheme on possible molecular targets for specific endpoints of TCDD toxicity. This scheme is drawn based on the results of studies cited in the text. Toxicity phenotypes were observed in a tissue- and time-specific manner and certain findings were based on *in vitro* results. For the pathways shown, “solid lines” are based on experimental evidence; “broken lines” are hypothesized. Representative abbreviations: PG, prostaglandin; PGDS, PGD synthase; TX, thromboxane.