

From the Institute of Environmental Medicine
Karolinska Institutet, Stockholm, Sweden

Influence of Oxidative Stress on Aryl Hydrocarbon Receptor Signaling

Afshin Mohammadi Bardbori



**Karolinska
Institutet**

Stockholm 2013

All previously published papers were reproduced with permission from the publisher.

Published by Karolinska Institutet. Printed by Universitetservice US-AB.

© Afshin Mohammadi Bardbori, 2013
ISBN 978-91-7549-163-9

تقدیم به معلمان بزرگ زندگیم پدر و مادر مهربانم که با ایثار ذره ذره
وجودشان بالیدن هر روزه مرا به نظاره نشسته‌اند.

ABSTRACT

The aryl hydrocarbon receptor (AHR), a multifunctional protein and a key regulator of drug metabolizing enzymes, belongs to the basic-helix-loop-helix (bHLH)/PAS (Per-Arnt-Sim) super-family of transcription factors. The AHR responds to exogenous and endogenous chemicals by induction or repression of a large number of genes involved in many physiological processes and normal development.

The diverse spectrum of AHR activators from well-known planar hydrophobic halogenated aromatic hydrocarbons (HAHs) to chemical compounds whose structure and physicochemical properties are very different from classical AHR ligands suggests that the AHR has a tremendously promiscuous ligand binding pocket. Due to the absence of a 3D structure of the ligand binding domain, promiscuity of the AHR has remained elusive. However, increasing experimental evidence indicate that the non-typical AHR ligands might activate the AHR signaling pathway indirectly by inhibiting the metabolic turnover of an endogenous ligand of the AHR. Therefore, the objective of this thesis was to characterize the inhibition of degradation of 6-formylindolo[3,2-*b*]carbazole (FICZ), the suggested natural high affinity AHR ligand, as a mechanism that could explain the earlier described agonistic properties of structurally very diverse AHR activators. The obtained results show that FICZ is a potent AHR agonist *in vitro* and *in vivo* which can distribute to the body through systemic circulation and induce cytochrome P450 1A1 (CYP1A1) the prototypical AHR target in various organs. The studies presented in this thesis demonstrate that if the metabolic clearance of FICZ is compromised, femtomolar concentrations of FICZ are sufficient to activate AHR signaling.

The AHR signaling pathway seems to be sensitive to oxidative stress but the redox regulation of AHR has not been well characterized. Studies on dioxin and other reactive oxygen species (ROS) producing agents have demonstrated that the AHR is a mediator of oxidative stress. Indeed, AHR works in close concert with the master regulator of antioxidant responses, nuclear factor (erythroid-derived 2)-like 2 (Nrf2). Multiple sources of ROS appear to be involved in modulating AHR signaling and probably via three major systems, microsomes, mitochondria and NADPH oxidase enzymes (NOXs). Furthermore, it has been observed that many environmental pollutants, including metals and other NOX-activators increase the levels of the diffusible molecule hydrogen peroxide (H_2O_2) and change the cellular redox status and thereby interfere with cell growth kinetics and the endogenous functions of the AHR. To increase the understanding of downstream adaptive responses to oxidative stress, including up-regulation of antioxidant genes and modulation of AHR signaling was another objective of this work. The findings demonstrate that superoxide anion ($\text{O}_2^{\cdot-}$) or H_2O_2 produced by NOXs can negatively and positively modulate the AHR signaling pathway. The importance of cellular redox levels which can influence endogenously activated AHR signaling broadens our earlier knowledge and explains why many oxidants behave both as AHR antagonists and agonists.

In summary, this thesis extends the mechanistic understanding of the promiscuity of AHR and provides important information with regard to the redox regulation of AHR endogenous signaling.

LIST OF PUBLICATIONS

- I. Wincent E, Bengtsson J, Mohammadi Bardbori A, Alsberg T, Luecke S, Rannug U, Rannug A. (2012). Inhibition of cytochrome P4501-dependent clearance of the endogenous agonist FICZ as a mechanism for activation of the aryl hydrocarbon receptor. *Proc Natl Acad Sci U S A*. 109(12):4479-84
- II. Mohammadi-Bardbori A, Bengtsson J, Rannug U, Rannug A, Wincent E. (2012). Quercetin, resveratrol and curcumin are indirect activators of the aryl hydrocarbon receptor (AHR). *Chem Res Toxicol*. 17;25(9):1878-84.
- III. Mohammadi-Bardbori A, Vikström Bergander L, Rannug U, and Rannug A. NADPH oxidase has a central role in aryl hydrocarbon receptor signaling and regulation of cell growth by arsenic, cadmium, mercury and nickel. (Manuscript)

Contents

1	INTRODUCTION.....	9
2	BACKGROUND.....	10
2.1	Structure of the AHR protein	10
2.2	Functions of the AHR protein.....	11
2.2.1	Involvement in metabolism of endogenous and exogenous chemicals	11
2.2.2	Physiological functions of the AHR	12
2.3	Activation of AHR signaling	12
2.3.1	AHR ligands.....	13
2.3.1.1	Xenobiotic compounds	13
2.3.1.2	Naturally-occurring compounds.....	14
2.4	Regulation of the AHR.....	15
2.4.1	AHR/CYP1A1-dependent auto-regulation	16
2.4.2	Repression by the AHR repressor	16
2.4.3	Regulation of AHR activity by modulation of receptor degradation.....	16
2.4.4	Crosstalk between the AHR and other pathways	16
2.4.5	Epigenetic effects on AHR target genes regulations.....	17
2.5	Modulation of AHR signaling by oxidative stress.....	17
2.5.1	Different sources of ROS	17
2.5.1.1	NADPH oxidase.....	18
2.5.2	Cellular defenses against ROS.....	19
2.5.2.1	Glutathione	19
2.5.2.2	The Nrf2-ARE signaling pathway	20
3	COMMENTS ON METHODOLOGY.....	22
3.1	Animals	22
3.2	Cell lines.....	22
3.3	Primary cells.....	22
3.4	Materials	22
3.4.1	AHR agonists	22
3.4.2	CYP1A1 inhibitors.....	23
3.4.2.1	Oxidants.....	23
3.4.2.2	Polyphenols	23
3.4.2.3	Metals and metalloids	25
3.5	Methods.....	25

3.5.1	Experimental designs	25
3.5.2	CYP1A1 inhibition assays	25
3.5.3	Analyses of cellular FICZ content	25
3.5.4	AHR activation assays	25
3.5.4.1	AHR activation in commercial and purified medium	25
3.5.5	Detection of oxidative stress	26
3.5.5.1	NADPH oxidase activity	26
3.5.5.2	GSH assay	26
3.5.5.3	Up-regulated antioxidant genes	26
3.5.6	NOX4 and Nrf2 silencing	26
3.5.7	Cell proliferation assay	26
4	AIMS OF PRESENT STUDY	27
5	RESULTS AND DISCUSSION.....	28
5.1	FICZ is a potent CYP1A1 inducer in vitro and in vivo	28
5.2	CYP1A1 inhibitors turn into AHR activators	28
5.2.1	Metabolic clearance of FICZ is inhibited by CYP1A1 inhibitors	28
5.2.2	CYP1A1 inhibitors activate AHR indirectly	29
5.3	Oxidative stress modulates AHR signaling	30
5.3.1	Oxidative stress antagonizes AHR activation	30
5.3.2	Oxidative stress up-regulates antioxidant genes.....	33
5.3.3	Preconditioning with oxidants leads to super-induction of CYP1A1	33
5.4	NADPH oxidase has a central role in regulating cell growth and aryl hydrocarbon receptor signaling.....	34
5.4.1	NOXs stimulate cell growth	34
5.4.2	NOXs have biphasic effects on AHR signaling pathway	34
6	CONCLUDING REMARKS AND FUTURE PERSPECTIVES.....	36
7	ACKNOWLEDGEMENTS.....	37
8	REFERENCES	39

LIST OF ABBREVIATIONS

AHR	aryl hydrocarbon receptor
AHRR	aryl hydrocarbon receptor repressor
AP-1	activator protein 1
ARE	antioxidant response element
ARNT	aryl hydrocarbon receptor nuclear translocator
As	arsenic
bHLH	basic helix-loop-helix
Cd	cadmium
CGD	chronic granulomatous disease
CUR	curcumin
CYP1A1	cytochrome P450 1A1
DPI	diphenyleneiodonium
DOUX	dual oxidases
EGF	epidermal growth factor
FICZ	6-formylindolo[3,2- <i>b</i>]carbazole
GSH	reduced glutathione
GSSG	oxidized glutathione
HaCaT	immortalized human keratinocyte cell line
HepG2-XRE-Luc	human hepatoma HepG2-derived cell line
Hg	mercury
HO-1	heme oxygenase 1
H ₂ O ₂	hydrogen peroxide
HSP90	heat shock protein 90 kDa
IL-1 α and β	interleukin-1 α and β
MNF	3'-methoxy-4'-nitroflavone
NF- κ B	nuclear factor kappa-light-chain-enhancer of activated B cells
Ni	nickel
NOXs	NADPH oxidase enzymes
Nrf2	nuclear factor (erythroid-derived 2)-like 2
PAS	Per-ARNT-sim
QUE	quercetin
RES	resveratrol
ROS	reactive oxygen species
TAD	transactivation domain
TCDD	2,3,7,8-tetrachlorodibenzo- <i>p</i> -dioxin
TGF α and β	growth factors α and β
TNF α	tumor necrosis factor α
Trp	tryptophan
UV	ultraviolet radiation (200-400nm)
XAP2	hepatitis B virus X-associated protein 2; AIP, aryl hydrocarbon receptor interacting protein
X-CGD	X chromosome- linked CGD human promyleocytic leukemia cell line
XRE/DRE	xenobiotic or dioxin response element

1 INTRODUCTION

The presence of aryl hydrocarbon receptor (AHR) homologues in early animals (about 570 million years ago) suggests that this protein family has ancient evolutionary origins (Peterson and Butterfield, 2005). The AHR was first identified in 1976 by Allan Poland, a physician who was interested in the etiology of chloracne among workers in a factory producing 2,4,5-trichlorophenol (Poland et al., 1976). The most toxic man-made anthropogenic compound, dioxin, was documented by German chemists as early as 1827 (reviewed in White and Birnbaum, 2009). The focus of research was aimed at identifying the biochemistry of AHR in 1980s. The ligand binding and DNA binding domains of the receptor were the most important findings during this period. In the early 1990s, the aryl hydrocarbon receptor nuclear translocator (ARNT), the nuclear partner of AHR, was identified. The crucial roles of AHR in physiology and biology were discovered by using knockout animals in the beginning of the 21st century (Gasiewicz and Henry, 2012). From current findings in the field of the AHR, it has become clear that AHR is more than a xenobiotic-interacting protein. The AHR turns out to be an important player in many physiological processes including cell cycle regulation, tumor suppression and immunity (reviewed by Barouki et al., 2007; Fujii-Kuriyama and Kawajiri, 2010).

The AHR can be activated by multitude of chemicals (Denison et al., 2011) and most of them do not fit into the ligand-binding pocket of the AHR. This thesis aimed at studying this claimed promiscuity of the AHR and to explain the earlier described agonistic and antagonistic properties of structurally very diverse molecules.

There is a paucity of data regarding how stress, in particular oxidative stress, influences the endogenously activated AHR signaling. It has been suggested that the AHR is a mediator of cellular stress responses (Matsumura, 2003; Matsumura and Vogel, 2006) and works in close concert with the Kelch-like ECH-associated protein 1 (Keap1)-nuclear factor erythroid 2-related factor 2 (Nrf2)-antioxidant response elements (ARE) pathway. Another aim of this study was therefore to fill this knowledge gap by studying the mechanisms of interaction between oxidants and ligand-mediated AHR signaling. The most interesting directions of future studies on the function of the AHR protein are expected to be:

- To learn the biology and physiology of the AHR in more detail
- To understand thoroughly the AHR endogenous signaling
- To know which human diseases may have AHR-dependent etiology and how to develop therapeutic treatments based on AHR modes of action
- To fully understand the molecular mechanism(s) of toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and other xenobiotics

2 BACKGROUND

2.1 STRUCTURE OF THE AHR PROTEIN

AHR along with its nuclear partner, ARNT, the aryl hydrocarbon receptor repressor (AHRR), the single-minded protein (Sim), the hypoxia inducible factors and many other proteins with roles for example in sensing oxygen, light and nitric oxide belong to the basic-helix-loop-helix (bHLH)/PAS (Per-Arnt-Sim) super-family of transcription factors (Gu et al., 2000; McIntosh et al., 2010). Among PAS proteins, only the AHR is a ligand-dependent transcription factor (Furness et al., 2007) and the AHR is not able to bind to a xenobiotic or dioxin response element (XRE/DRE) in the promoter region of target genes without activation by a ligand. The AHRR has been described as an inducible protein that competes with the AHR for dimerization with ARNT for binding to DNA (Mimura et al., 1999). The AHRR binding to XRE/DRE has also been reported to be independent from ARNT heterodimerization (Hahn et al., 2009).

As shown in figure 1, the N-terminal region of AHR comprises a bHLH domain followed by PAS A and B motifs. The C-terminal region consists of a transactivation domain (TAD) (Jain et al., 1994). AHR/ARNT heterodimerization, DNA binding and recognition are mediated by the TAD domain (Reyes et al., 1992; Dolwick et al., 1993; Swanson, 2002). A nuclear localization signal (NLS) and nuclear export signal (NES) overlap with the DNA binding domain (Ikuta et al., 1998). Another NES overlaps with the PAS domain (Berg and Pongratz, 2001). Unmasking of NLS by the release of heat shock protein (HSP90) is required for localization of AHR into the nucleus (Ikuta et al., 1998) and translocation of AHR is important for AHR mediated responses. Nuclear export of AHR is also important for AHR proteasomal degradation (Pollenz et al., 1999). Interaction with chaperones, ligand recognition and AHR/ARNT heterodimer formation are mediated by the PAS domain. It consists of two regions of PAS A and B with high sequence homology (Dolwick et al., 1993; Fukunaga and Hankinson, 1996; Taylor and Zhulin, 1999; Gu et al., 2000). The TAD domain consists of three acidic, glutamine-rich and prolin-serin-threonin sub-domains (Ma et al., 1995). Each sub-domain has its own transactivation activity and all sub-domains show synergic activities in combination (Whitelaw et al., 1994). An inhibitory domain (ID) between the PAS-B domain and the TAD domain consisting of 81 residues was identified with inhibitory effects on TAD domain in the absence of an agonist (Ma et al., 1995).

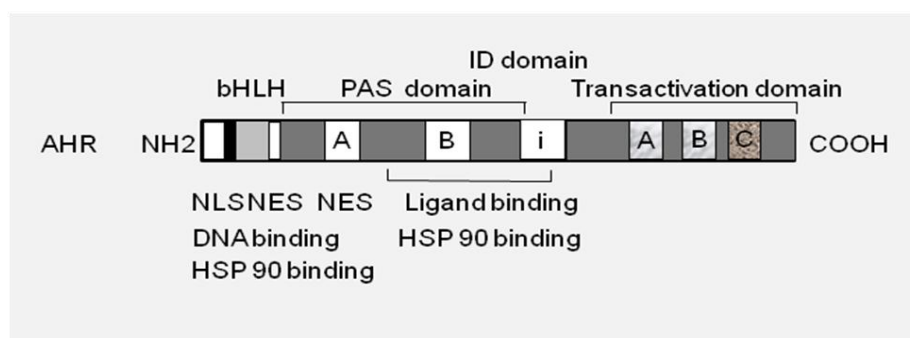


Figure 1. Functional domains of the AHR. Figure representing basic regions for DNA and ligand binding, dimerization and transactivation.

2.2 FUNCTIONS OF THE AHR PROTEIN

2.2.1 Involvement in metabolism of endogenous and exogenous chemicals

The AHR is involved in up-regulating a large number of xenobiotic metabolizing enzymes including phase I and II metabolizing enzymes and phase III transporters in response to noxious chemicals and numerous endogenous molecules. The cytochrome P450 monooxygenase 1 proteins (CYP1s), glutathione S-transferases (GSTs) and UDP-glucuronosyltransferases (UGTs) are examples of AHR-dependent metabolizing enzymes. One role of bio-transformation is to terminate the biological or toxic effects of lipophilic chemicals by incorporating a functional groups such as -OH, -NH, -SH or -COOH in the molecule to make them more water soluble (Parkinson, 1996). Another role is to form biologically active products such as arachidonic acid metabolites (prostaglandins). However, in some cases oxidative metabolism of xenobiotics by CYP enzymes may lead to the formation of reactive intermediates and increase the toxicity (reviewed by Shimada, 2006). Involvement of CYPs in both detoxification of xenobiotics and formation of reactive intermediates that is associated with toxicity, mutagenesis and carcinogenesis has been investigated in knockout animal models. CYP1A1 (-/-) knockout mice exposed daily to benzo[a]pyrene (BaP) were dying within 30 days, whereas CYP1A1 (+/+) wild type mice didn't exhibit any signs of toxicity. BaP- DNA adducts were higher in knockout animals compare to wild type mice (Uno et al., 2004). The toxicity of oral BaP in double-knockout mice for both CYP1A1 and 1B1 was also higher than in the other genotypes investigated and the authors concluded that CYP1A1 mediates detoxification of BaP by speeding up the metabolism and excretion (Uno et al., 2006).

The most common reactions catalyzed by CYPs are insertion of one oxygen molecule into the lipophilic substrate (reviewed by Hollenberg, 2002). The reaction can be summarized as follows: 1) binding of substrate to the active site of enzyme 2) reduction of prosthetic heme iron from ferric (Fe^{+3}) to ferrous (Fe^{+2}) 3) binding of oxygen to ferrous molecule 4) transferring electron from NADPH via cytochrome P450 reductase to ferrous-oxygen-substrate complex 5) formation of activated oxygen intermediate and water 6) insertion of activated oxygen intermediate into the substrate to make an oxygenated product. In this reaction, several steps including steps number one, three and six are more susceptible for inhibition by CYPs inhibitors. In general, chemicals that can inhibit the reactions before the formation of ferrous-oxygen-substrate complex are reversible inhibitors and are simply divided into competitive, noncompetitive and uncompetitive inhibitors. Those inhibitors which act after the formation of ferrous-oxygen-substrate complex are either quasi-irreversible or irreversible inhibitors. The type of inhibition of quasi-irreversible or irreversible inhibitors is dose-time-dependent whilst reversible inhibitors only show dose-dependent inhibition manner (reviewed in Hollenberg, 2002).

In phase II of bio-transformation conjugation with glucuronic acid, sulfate, or acetic acid is catalyzed by UDP-glucuronosyl transferases (UGTs), sulfotransferases (SULTs) and N-acetyltransferases (NATs) respectively (Negishi et al., 2001; Wells et al., 2004; Sim et al., 2008; Omiecinski et al., 2010). The role of phase III is to transport drugs and xenobiotics via membrane transporters such as ATP-binding cassette family (ABC) of proteins across the cellular membranes (Omiecinski et al., 2010). Expression of ATP-dependent transporters were shown to be induced by AHR ligands (Maher et al., 2006).

2.2.2 Physiological functions of the AHR

The abnormal phenotypes of AHR deficient animals clearly have shown that AHR is an important protein for proper development of liver (Fernandez-Salguero et al., 1995; Schmidt et al., 1996), ovaries (Benedict et al., 2000), heart (Fernandez-Salguero et al., 1996; Mimura et al., 1997; Lahvis and Bradfield, 1998), as well as cardiovascular (Lahvis et al., 2000; Vasquez et al., 2003; Lahvis et al., 2005) and reproductive systems (Baba et al., 2005). The role of AHR in cell proliferation and apoptosis is not well-known. In AHR-defective cells, the rate of cell growth seems to be slower than those with normal phenotypes (Ma and Whitlock, 1996; Weiss et al., 1996). The same observation has been made in mouse embryonic fibroblasts derived from AHR null mice (Elizondo et al., 2000). The AHR may be involved in apoptosis and cell death and it has been suggested that the AHR may bind to the transcription factor E2F1 and inhibit apoptosis (Marlowe et al., 2008). In addition to the suggested physiological role of AHR in cell cycle progression and normal development, AHR plays important roles in the normal function of immune and endocrine systems, circadian rhythm, skin physiology, hematopoiesis, and stem cell expansion (Schmidt et al., 1996; Baba et al., 2005; Fritsche et al., 2007; Mukai and Tischkau, 2007; Esser et al., 2009; Boitano et al., 2010; Casado et al., 2010).

2.3 ACTIVATION OF AHR SIGNALING

The unliganded AHR resides in the cytoplasm in complex with two molecules of chaperons, HSP90, a low molecular weight AHR interacting protein (AIP) also called XAP2 or ARA9 and p23. Interaction with HSP90 seems to be essential for ligand and DNA binding of the receptor (Perdew, 1988; Wilhelmsson et al., 1990; Pongratz et al., 1992; Carver et al., 1994; Ma and Whitlock, 1997). The AHR binds to the middle part of HSP90 (amino acids 272-617) (Meyer and Perdew, 1999) and its interaction with HSP90 and co-chaperones seems to be central to the stability of the AHR complex in the cytoplasm (Song and Pollenz, 2002). On the other hand, protein-protein interaction is a key component of the folding machinery. The chaperons make the receptor more sensitive to its ligands (Carver and Bradfield, 1997; Carver et al., 1998; Kazlauskas et al., 1999; Meyer and Perdew, 1999; Bell and Poland, 2000; Meyer et al., 2000). p23 enhances the ligand binding activity and transcription activity of AHR and increases the ability of AHR to bind to XRE/DRE (Young et al., 2001; Cox and Miller, 2002; Shetty et al., 2003). Upon ligand binding and activation, AHR translocates into the nucleus, detaches from chaperons, and dimerizes with its nuclear partner ARNT (Carver et al., 1994). Nuclear translocation is followed by a reduction in the size of the receptor, which can be the result of detachment from chaperons and dimerization with ARNT (McGuire et al., 1994). Studies on ARNT deficient mouse hepatoma Hepal4 cells showed that ARNT is required for AHR binding to specific response elements in DNA (XRE/DRE) (Probst et al., 1993; Whitelaw et al., 1993). The AHR/ARNT heterodimer complex binds to XRE/DRE and up-regulates a battery of genes involved in biotransformation of xenobiotic and endobiotic compounds and hundreds of other genes (reviewed by Nebert and Dalton, 2006) (Figure 2).

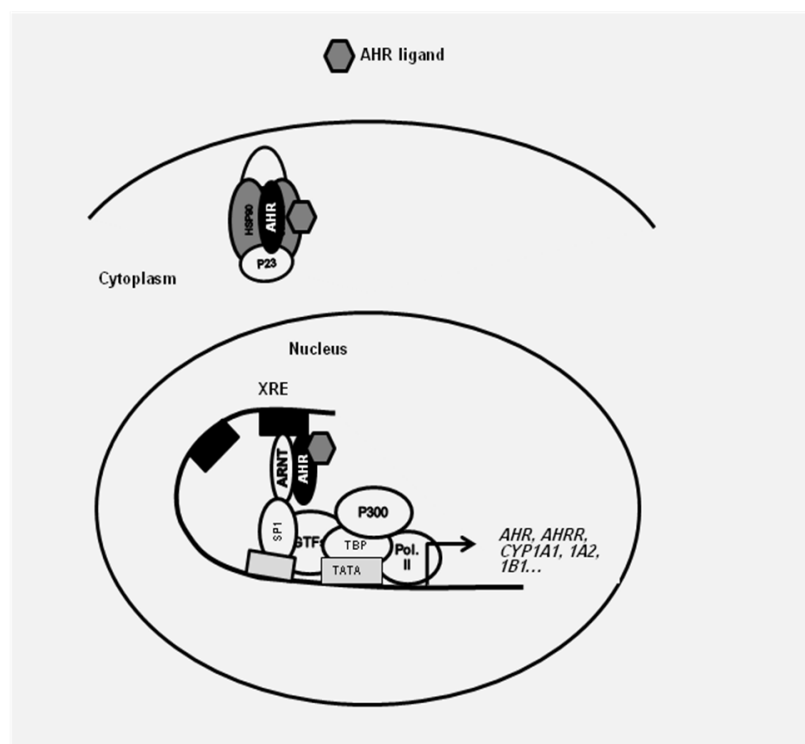


Figure 2. Schematic structure of the AHR and molecular mechanism of AHR activation by an AHR ligand (based on Fujii-Kuriyama & Mimura, 2005)

2.3.1 AHR ligands

The physiochemical properties of AHR ligands have been tested for more than 30 years. Structure-activity relationship studies suggest that hydrophobic and planar compounds are well fitted to the ligand-binding pocket of AHR. According to molecular modeling studies, the ligand-binding pocket of AHR doesn't accept a ligand bigger than 14x12x5 Å (Waller and McKinney, 1995). However, a large number of natural, endogenous and synthetic compounds that do not fit into the described pocket can activate AHR signaling (reviewed in Denison et al., 2002). This suggests that the AHR has a more promiscuous ligand binding pocket. Due to the absence of 3D crystal/NMR/ X ray structure of the ligand binding domain (LBD) the promiscuity of the ligand-binding pocket of AHR remains elusive.

2.3.1.1 Xenobiotic compounds

Environmental pollutants such as halogenated aromatic hydrocarbons (HAHs) and polycyclic aromatic hydrocarbons (PAHs) are known as synthetic ligands of the AHR (reviewed in Denison et al., 2002; Denison and Nagy, 2003; Fujii-Kuriyama and Mimura, 2005). Among xenobiotic compounds, TCDD (Figure 3) has the highest affinity for binding and activation of the AHR. Among the described xenobiotic ligands, TCDD has the highest toxicity and bio-accumulation with the half-life in human of about 7.5 years (Whitlock, 1999; Van den Berg et al., 2006). Induction of CYP1A1 by TCDD in the body is highly persistent due to the long halftime of TCDD (Ma and Baldwin, 2000).

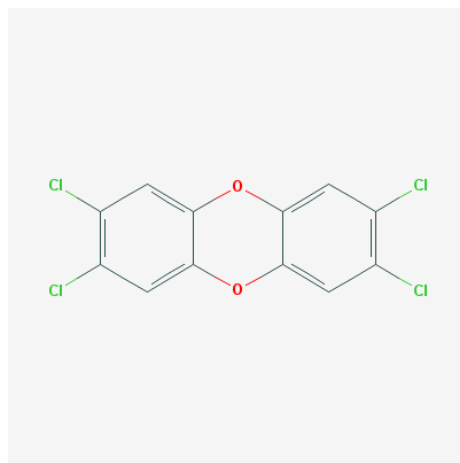


Figure 3. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)

2.3.1.2 Naturally-occurring compounds

In addition to synthetic ligands, numerous naturally occurring and endogenous compounds are able to bind and activate the AHR (Denison and Nagy, 2003; Nguyen and Bradfield, 2008). Indole-3-carbinol derivatives such as indolo[3,2-*b*]carbazole (ICZ) (Bjeldanes et al., 1991; Chen et al., 1995), phytochemicals including alkaloids and flavonoids and heme metabolites have been introduced as agonists of AHR (Gillner et al., 1989; Gasiewicz et al., 1996; Adachi et al., 2001). Arachidonic acid products (prostaglandins and lipoxins A4) (Schaldach et al., 1999; Seidel et al., 2001), and tryptophan (Trp) derivatives (tryptamine, indole acetic acid, indigo and indirubin) (Heath-Pagliuso et al., 1998; Adachi et al., 2001) are natural activators of AHR signaling.

A large body of evidence has pointed to the role of an endogenous ligand of the receptor for activation of AHR in the absence of exogenously added AHR ligands (Paine, 1976; Hankinson et al., 1985; Sadek and Allen-Hoffmann, 1994; Singh et al., 1996; Crawford et al., 1997; Chang and Puga, 1998; Chiaro et al., 2007). Most of the suggested endogenous ligands for the AHR so far have exhibited low affinity for binding and activation of the receptor. Among all tested compounds a photoproduct of the amino acid Trp, 6-formylindolo[3,2-*b*]carbazole (FICZ), is receiving increasing attention (Ma, 2011; Bock, 2012) (Figure 4). FICZ exerts several unique properties, which make it a possible endogenous ligand of the receptor. It exhibits the highest affinity for binding and activation of the receptor (Rannug et al., 1987; Nguyen and Bradfield, 2008). FICZ can be formed in aqueous solutions containing Trp when exposed to visible and UV light (Oberge et al., 2005; Diani-Moore et al., 2006; Wincent et al., 2009) and FICZ has been found in human skin (Magiatis et al., 2013) and its sulfate conjugates have been detected in human urine (Wincent et al., 2009). Furthermore, FICZ is an excellent substrate for CYP1A1 (Wincent et al., 2009) and was suggested to contribute to the auto-regulatory feedback control of AHR signaling.

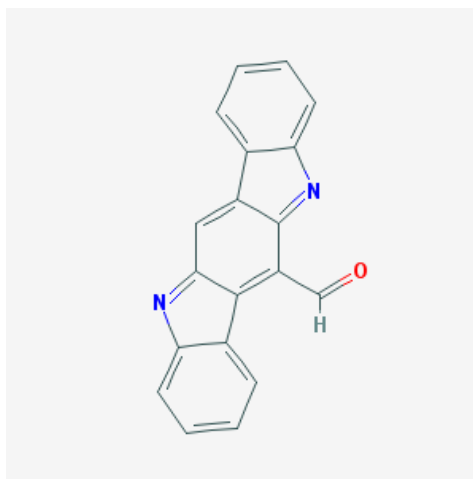


Figure 4. 6-formylindolo[3,2-*b*]carbazole (FICZ)

2.4 REGULATION OF THE AHR

Because the AHR is a multifunctional protein that plays a fundamental role in normal development and cell physiology, there are multiple mechanisms designed to suppress sustained AHR activation. These include an AHR repressor, proteasomal degradation of the AHR, inducible CYP1A1 mediated degradation of ligands and epigenetic mechanisms. This shows that tight regulation of AHR signaling pathway is very important (Figure 5).

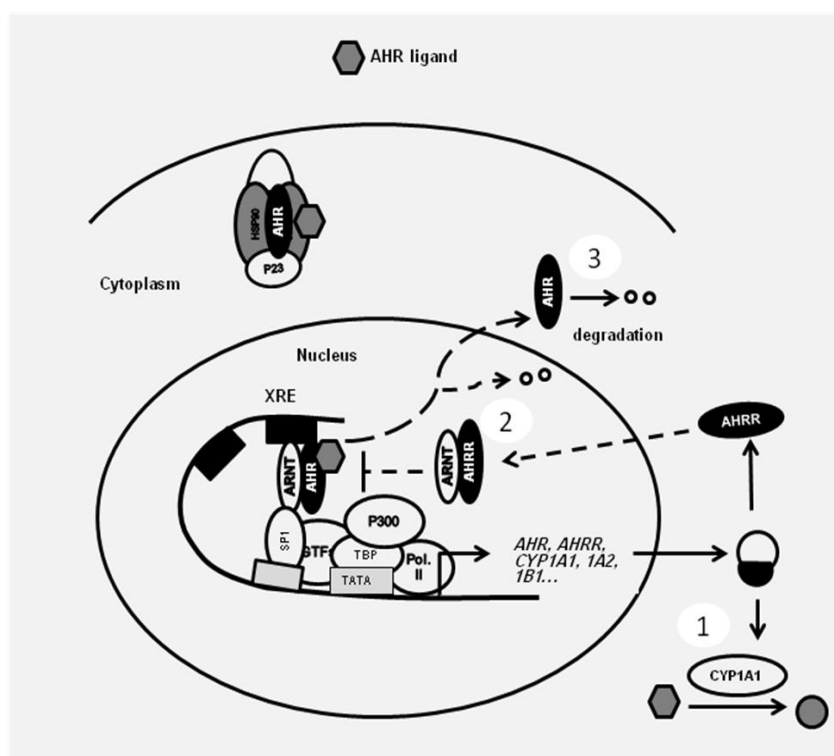


Figure 5. AHR activation can be regulated by 1) AHR/CYP1A1/FICZ-dependent auto-regulation 2) AHRR/ARNT complex formation 3) proteasomal degradation of AHR (based on Fujii-Kuriyama & Mimura, 2005)

2.4.1 AHR/CYP1A1-dependent auto-regulation

The AHR induces the expression of metabolizing enzymes in particular CYP1A1, after exposure to AHR ligands. CYP1A1 regulates AHR activity by elimination of the ligands and thereby decreases the duration of exposure. In this way the AHR/CYP1A1 auto-regulation feedback causes transient activation of AHR (Nebert and Dalton, 2006; Chiaro et al., 2007).

2.4.2 Repression by the AHR repressor

The AHRR was first identified to repress the AHR activity by competing with the AHR/ARNT heterodimer formation (Mimura et al., 1999; Baba et al., 2001). It is becoming more apparent that the AHRR/ARNT heterodimer doesn't competitively displace the AHR protein from AHR/ARNT heterodimer and AHRR DNA binding seems to be independent to ARNT (Hahn et al., 2009). AHRR plays important roles in repression of AHR mediated responses in some cell lines such as human dermal fibroblasts (Haarmann-Stemmann et al., 2007). However, expression of AHRR is cell-, tissue-, and species specific. In addition, repression of AHR by AHRR in *in vivo* studies remains elusive (Haarmann-Stemmann and Abel, 2006). Furthermore, AHRR also can repress the activity of the other transcription factors such as estrogen receptor alpha (Ohtake et al., 2003).

2.4.3 Regulation of AHR activity by modulation of receptor degradation

Levels of AHR can be regulated by the rate of expression and the rate of degradation of the protein. Expression of AHR metabolizing enzymes and AHR protein is under the control of the AHR signaling pathway and the keap1-Nrf2-antioxidant response element (Shin et al., 2007). The half-life of liganded-AHR is much shorter than unliganded-AHR, indicating that the ligand-activated AHR undergoes degradation after activation and binding to DNA (Ma and Baldwin, 2000). The down regulation of AHR through the 26S proteasomal pathway starts by labeling of the protein with a polypeptide, ubiquitin, then, the protein is recognized by the 26S proteasome and is degraded into short peptides (Davarinos and Pollenz, 1999). There seems to be a regulatory feedback control between AHR transcriptional activity and degradation (Davarinos and Pollenz, 1999; Roberts and Whitelaw, 1999; Pollenz, 2002; Song and Pollenz, 2003).

2.4.4 Crosstalk between the AHR and other pathways

It has been observed that TCDD has anti-estrogenic effects (Kociba et al., 1978). Crosstalk between the AHR signaling pathway and retinoic acid receptor (Wanner et al., 1995; Lorick et al., 1998), estrogen receptor (Spink et al., 1990; Heimler et al., 1998; Tian et al., 1998), growth factors and cytokine signaling (Haarmann-Stemmann et al., 2009), transcription factor SP1 (Fisher et al., 1990) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) (Tian et al., 1999; Kim et al., 2000) has been reported. Several protein kinases such as protein kinase C (PKC) and protein tyrosine kinase (PTK) have been implicated in the regulation of AHR by phosphorylation of its C-terminal region (Gradin et al., 1994; Mahon and Gasiewicz, 1995). NF- κ B activators such as bacterial endotoxins and pro-inflammatory cytokines are reported repressors of CYP1A1. Other authors suggested competition for common

co-activators (p300) to be a mechanism for suppression of CYP1A1 by NF- κ B (Tian et al., 1999; Ke et al., 2001). The presence of multiple copies of XRE/DREs within the promoters of human Nrf2 genes and induction of Nrf2 and Nrf2 target genes by TCDD indicate that the AHR interacts with Nrf2 via two possible mechanisms. First, the AHR ligands such as TCDD directly up-regulate expression of Nrf2 and phase II metabolizing enzymes and second, AHR/ARNT/Nrf2 engage in the mutual binding to XRE/DRE (Ma et al., 2004; Yeager et al., 2009). Moreover, it has been shown that the level of AHR mRNA in Keap-1 knockout mice is higher than in normal animals suggesting a direct crosstalk between AHR and Nrf2 signaling pathways (Shin et al., 2007).

2.4.5 Epigenetic effects on AHR target genes regulations

DNA methylation and chromatin remodeling by histone acetylation and de-acetylation are epigenetic mechanisms shown to take part in regulation of human AHR target genes after exposure to exogenous and endogenous chemicals. Histone acetylation and de-acetylation modify the structure of chromatin to be accessible or inaccessible for the transcription factors and co-activators/co-repressors. Recruitment of histone acetylase (HAT), co-activators such as p300, and steroid receptor co-activator 2 (SRC-2) to the enhancer and promoter of both CYP1A1 and CYP1B1 genes are required for proper transcription activity (Taylor et al., 2009). Also different patterns of DNA methylation of AHR target genes provide valuable information with regard to the epigenetic modification of AHR signaling. It has for example been observed that CpG dinucleotides in the promoter and enhancer of the CYP1B1 gene exhibit DNA methylation in some cell lines while CYP1A1 is not fully methylated and is inducible (Han et al., 2006; Habano et al., 2009; Beedanagari et al., 2010).

2.5 MODULATION OF AHR SIGNALING BY OXIDATIVE STRESS

The dual function of reactive oxygen species (ROS) in biological systems is well established for several years. Regulatory events for ROS production mainly by the NADPH oxidase enzyme (NOX) family and maintenance of redox homeostasis by the activation of redox sensitive transcription factors such as Nrf2, the activator protein 1 (AP-1) and NF- κ B are key events in the normal physiology of living cells. Diffusible H₂O₂ can act as a fundamental signaling molecule in activation/inhibition of several transduction signaling pathways. ROS sometimes play opposing roles in cellular signaling. For instance, activation of extracellular signal-regulated kinase (ERK1/2) by ROS can promote cell survival whereas activation of c-Jun N-terminal (JNK)/stress-activated protein kinase can induce apoptosis (reviewed in Groeger et al., 2009).

The redox regulation of AHR signaling is not well characterized but it has been shown previously that the sulfhydryl groups in the AHR complex and the DNA binding domain of the AHR are sensitive to oxidative stress (Denison et al., 1987; Pongratz et al., 1992; Ireland et al., 1995; Xu et al., 1998; Cumming et al., 2004).

2.5.1 Different sources of ROS

Superoxide anion (O₂⁻) is a product of the one-electron reduction of an O₂ molecule. In mammalian cells, ROS can be generated from different sources such as mitochondria (mainly from complex I and III), endoplasmic reticulum (microsomes fractions),

peroxisomes, NO synthases and lipoxygenases in cytosol and NOX enzymes in the plasma membrane (Halliwell and Cross, 1994). ROS also can be produced by xanthine oxidase in extracellular spaces (Brown and Borutaite, 2012).

2.5.1.1 *NADPH oxidase*

Activation of NOX enzymes in macrophages and phagocytes produces millimolar concentrations of H_2O_2 to kill bacteria and other pathogens (Rhee, 2006). In contrast, activation and expression of NOXs in non-phagocytes is a highly regulated process that is involved in many cellular signaling pathways (reviewed in Droge, 2002). The acute activation of NOX family members (NOX1-4, NOX5 and the dual oxidases DUOX1 and DUOX2) are regulated either by intracellular levels of calcium or phosphorylation of regulatory subunits. Non-phagocyte NOXs can be activated by different stimuli such as transforming growth factors α and β (TGF α and β), interleukin-1 α and β (IL-1 α and β), epidermal growth factor (EGF), tumor necrosis factor α (TNF α), mechanical forces stimulation (in endothelial cells), changes in oxygen tension, hormones or local metabolic changes (reviewed by Jiang et al., 2011). The glycosylated catalytic moiety of NOX enzymes, gp91^{phox} consists of a flavin-adenine dinucleotide (FAD) sequestered in the cell membrane in association with its trans-membrane partner p22^{phox} (Figure 6). Complex formation with the p22^{phox} subunit is needed for NOX activation (Sumimoto et al., 1996; Ambasta et al., 2004; Martyn et al., 2006). This complex is referred to as cytochrome b558. Phosphorylation of the P47^{phox} subunit and recruitment of a small Rho GTPase protein (Rac) result in release from auto-inhibition, translocation to the membrane and binding to p22^{phox} (reviewed in Groeger et al., 2009). The N-terminal of NOXs comprises six predicted α -helic regions for electron transferring (reviewed by Lambeth et al., 2007). The maximal activity of NOXs can be determined by the activation state of regulatory subunits and expression of NOX proteins. The highest level of NOX1 protein is found in colon and to some extent in other tissues and cell lines such as vascular smooth muscles and pulmonary epithelial cell line. NOX2 and NOX3 are expressed in mature myeloid cells and in the inner ear respectively (reviewed in Lambeth et al., 2007). Among NOX families, NOX4 is constitutively active (reviewed by Droge, 2002) and can be activated by various types of activators. Acute NOX4-dependent generation of ROS by stimulation with insulin (after five min) and lipopolysaccharide (after 30 min) has been reported (Mahadev et al., 2004; Park et al., 2004; Lambeth et al., 2007). Expression of NOX4 is predominantly observed in the kidney and liver and moderate levels of NOX4 are expressed in other organs (Cheng et al., 2001; Mahadev et al., 2004). High levels of NOX5 are expressed in spleen, testis and vascular smooth muscle (reviewed in Lambeth et al., 2007). DUOX1 and DUOX2 are found in the thyroid (De Deken et al., 2002). A compensatory expression mechanism between NOX4 and NOX2 has been observed in some cell lines (Petry et al., 2006; Pendyala et al., 2009).

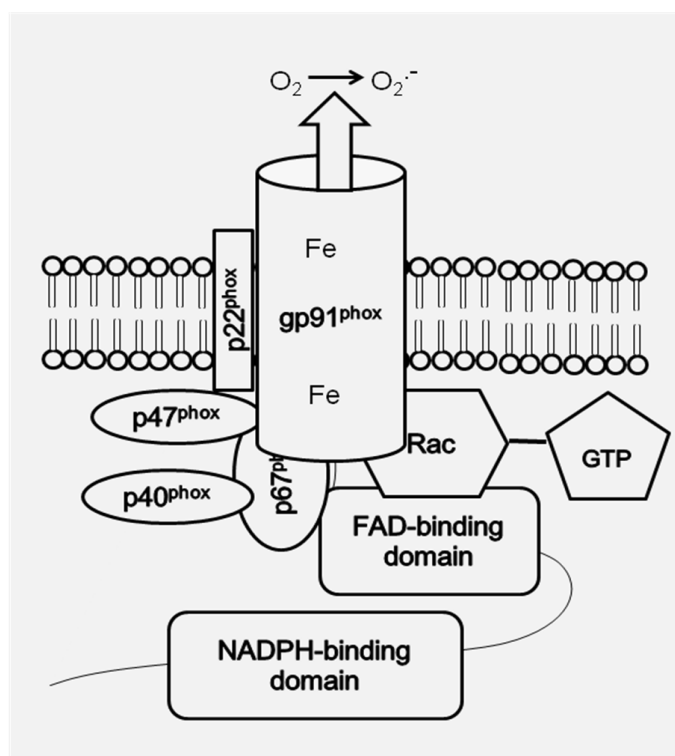


Figure 6. Schematic representation of NOX1-4 protein structure (based on Groeger et. al., 2009).

2.5.2 Cellular defenses against ROS

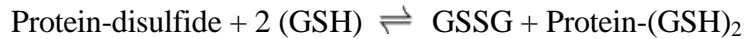
$O_2^{\cdot -}$ is an unstable radical species with a very short half-life. It can be converted to non-radical species such as H_2O_2 enzymatically by superoxide dismutase (SOD) or spontaneously in cells. H_2O_2 in the presence of reduced transition metals can be converted into the very reactive and harmful hydroxyl radical ($HO\cdot$). To neutralize and use the advantage of ROS, antioxidants play important roles in living cells (Halliwell, 2007). Some cellular antioxidants are present at low levels but work with high efficiency (catalase and glutathione peroxidase) and some are present in high levels but work with low efficiency (free amino acids and proteins). Living organisms also use nonenzymatic antioxidants such as vitamins and glutathione to scavenge ROS (reviewed by Droge, 2002). In mammalian cells SOD, catalase and glutathione peroxidase convert H_2O_2 to water and oxygen.

2.5.2.1 Glutathione

The tripeptide γ -L-glutamyl-L-cysteinylglycine or glutathione is an abundant non-protein antioxidant present in all mammalian cells at 1-10 mM concentration. Glutathione exists in both reduced (GSH) and oxidized (GSSG) form. More than 98% of glutathione at normal physiological conditions is present in the reduced form (Akerboom et al., 1982). Unusual linking between glutamate and cysteine (γ -carboxyl instead of α - carboxyl) makes it resistant to degradation by intracellular enzymes. Glutathione is degraded by an extracellular enzyme, γ -glutamyltranspeptidase (GGT), on the surface of certain cell types (Meister and Anderson, 1983; Lu, 2012). Glutathione is synthesized in two ATP-dependent reactions. First, formation of γ -glutamylcysteine by a glutamate cysteine ligase (GCL) and second, the addition a

glycine amino acid to the γ -glutamylcysteine by glutathione synthetase (GS) forms the tripeptide, γ -L-glutamyl-L-cysteinylglycine. GCL is composed of catalytic and modifier subunits (GCLC and GCLM) and is a crucial enzyme in GSH homeostasis. Glutathione synthesis can be limited by the availability of cysteine amino acid. It has been shown that the reducing agent dithiothreitol is able to reversibly inactivate GCL whilst oxidative stress facilitates the holoenzyme formation (Seelig et al., 1984; Franklin et al., 2009). GSH plays several important functions in living cells including 1) detoxification of electrophiles enzymatically in a reaction catalyzed by glutathione-S-transferases 2) scavenging of ROS in a reaction catalyzed by glutathione peroxidases 3) regulation of redox-dependent cell signaling pathways by redox-sensitive transcription factors and 4) serving as a continuous source of cysteine (Meister and Anderson, 1983; DeLeve and Kaplowitz, 1991; Lu, 2012). In the reaction catalyzed by glutathione peroxidase, a sulfhydryl moiety of the cysteine residue of GSH donates a reducing equivalent to neutralize H_2O_2 to water. In this reaction two molecules of GSH are oxidized to GSSG. GSSG can be reduced back to GSH by glutathione reductase using NADPH as an electron donor.

GSH plays an important role in maintenance of the cellular redox state that is crucial for regulation of signal transduction pathways and gene expression through activation of transcription factors. The intracellular redox state of the cells is maintained by two systems, the GSH/GSSG ratio that is present at millimolar and thioredoxin (Trx) at micromolar concentrations. The steady-state balance of GSH and GSSG during oxidative stress conditions is different. The redox state of cells is determined in a reversible reaction catalyzed by thiol-transferase as follows (reviewed in Lu, 1999):



A convenient expression has also been introduced for the calculation of GSH redox potential according to the Nernst equation as follows:

$$Eh = E^o + (RT/nF) \ln([\text{acceptor}]/[\text{donor}])$$

In the above equation, Eh (mV) at defined pH is calculated relative to a standard hydrogen electrode. In this expression R , T , n and F are gas constant, absolute temperature, the number of electrons transferred and Faraday's constant respectively (see a review by Jones, 2002). Extracellular and intracellular redox potentials provide useful indicators in association with redox-dependent cellular signaling. For instance, the intracellular GSH/GSSG potential redox is reduced during proliferation and becomes oxidized in differentiation and apoptosis (from -260mV to -150mV). The extracellular Cys/CySS redox follows the same pattern (Moriarty-Craige and Jones, 2004). While more than 98% of cellular glutathione under normal physiological conditions is present in the reduced form (Akerboom et al., 1982), any changes in the intracellular GSH level can be useful for predicting cellular signaling.

2.5.2.2 The Nrf2-ARE signaling pathway

Nrf2 is a redox sensitive transcription factor that controls the expression of a group of enzymes protecting against reactive oxygen intermediates. Nrf2-deficient mice are more susceptible to developing different types of cancer and chronic diseases (Ramos-Gomez et al., 2001; Rangasamy et al., 2004; Khor et al., 2006). Under non-stress physiological conditions, Nrf2 is sequestered by its cysteine-rich, Kelch domain-

containing partner (Keap1) in the cytoplasm. The Keap1-Nrf2 complex is rapidly degraded by the ubiquitin proteasome pathway after oxidation (Itoh et al., 2003). Both the N-terminal Broad complex, Tramtrack and Bric-à-Brac (BTB) and intervening-region (IVR) domains of Keap1 are required for Nrf2 degradation and a subunit of E3 ubiquitin ligase (Cul3) interacts with the IVR domain of Keap1 (Kobayashi et al., 2004). Overexpression of Cul3 leads to rapid degradation of Nrf2 and this has been shown to enhance the risk of breast cancer (Loignon et al., 2009). **The Nrf2 pathway can be activated not only by chemical compounds with the capacity to undergo redox cycling or by reactive oxygen intermediates but also by compounds that have the ability to react with sulfhydryl groups.** Thus, alterations in the glutathione cellular levels and the redox state of cells can trigger the Nrf2 signaling pathway (Talalay et al., 2003). Modification of two cysteine (C273 and C288) residues in the IVR domain by ROS and formation of intermolecular disulfide bridges leads to the release of Nrf2 and its translocation to the nucleus (Dinkova-Kostova et al., 2002; Kobayashi et al., 2004; Wakabayashi et al., 2004) where it dimerizes with Maf, one of the small bZIP proteins. Activated Nrf2 binds to the antioxidant response element (ARE) and causes ARE-dependent transcription of antioxidant genes such as GST, NAD(P)H:quinone oxidoreductase 1 (NQO1), heme oxygenase 1 (HO-1), ferritin, GCL, glutathione reductase and aldehyde dehydrogenase (Hayes and McLellan, 1999; Talalay et al., 2003; Kensler et al., 2007) (Figure 7). It has been suggested that Keap1 regulates Nrf2 activity but the mechanism of stabilization of Nrf2 activity is not well known. Nrf2 has been found to be an unstable protein with a short half-life ($t_{1/2}$ around 15 min). After activation with ROS, Cul3 targets Nrf2 for ubiquitin-dependent degradation by the 26S proteasome (Nguyen et al., 2003; Nguyen et al., 2009).

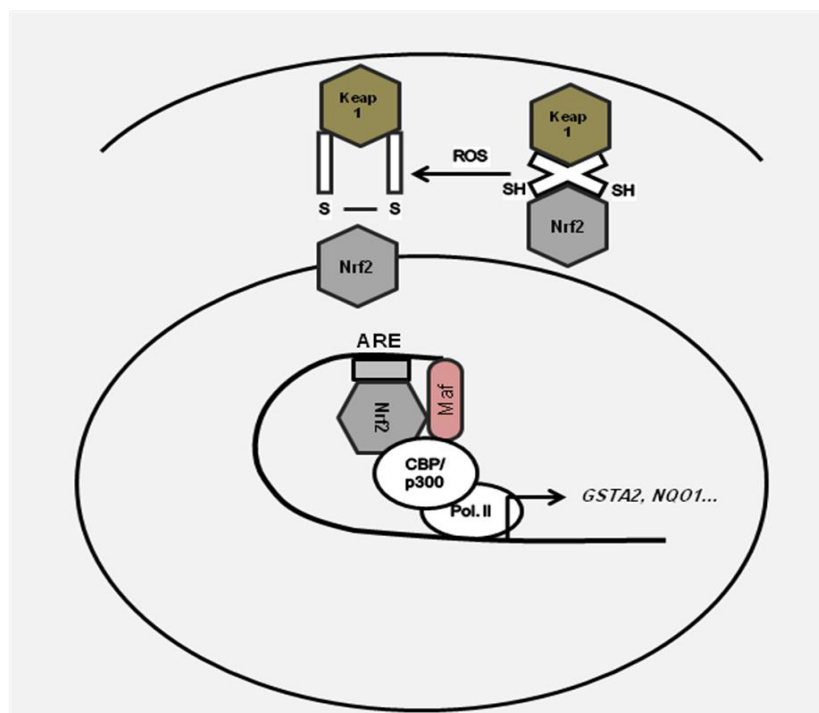


Figure 7. General scheme for the activation of the Keap1-Nrf2-ARE signaling pathway. ROS disrupts the Keap1-Nrf2 association leading to formation of intermolecular disulfide bridges and accumulation of Nrf2 in nucleus where it dimerizes with small protein Maf, binds to ARE and stimulates expression of ARE responsive genes.

3 COMMENTS ON METHODOLOGY

3.1 ANIMALS

In order to determine whether FICZ can be distributed to the body through the systemic circulation, experiments were carried out on female C57BL/6J mice (Paper I). The experiment was conducted in accordance with the regulations for animal experimentation at Karolinska Institutet and was preapproved by the Ethical Committee on Animal Experimentation in Stockholm.

3.2 CELL LINES

The immortalized human keratinocyte cell line (HaCaT) was kindly provided by N. E. Fusenig (DKFZ, Heidelberg, Germany). HaCaT cells were grown at a high density to elucidate the AHR activation by different AHR activators and CYP1A1 inhibitors (Paper I, II and III). Higher expression of differentiation markers (involucrin and transglutaminase) and AHR protein was documented when HaCaT cells were grown at high density (Paper I).

The human hepatoma HepG2-derived cell line HepG2-XRE-Luc, containing a pTX.DIR- luciferase reporter under the control of two XRE/DRE sequences of the rat CYP1A1 gene, was kindly provided by K. Gradin, Karolinska Institutet. HepG2-XRE-Luc was used to investigate CYP1A1 reporter gene activation in response to different types of AHR activators and CYP1A1 inhibitors (Paper II and III).

The human X chromosome-linked chronic granulomatous disease (X-CGD) derived-PBL-985 cell line (X-CGD cells) carrying a mutated gp91^{phox} gene (Zhen et al., 1993) and the X-CGD cell line re-transfected with gp91^{phox} (X-CGD-gp 91^{phox} cells), originally constructed by M.C. Dinauer (Ding et al., 1996), were kindly provided by B. Fadeel, Karolinska Institutet. The X-CGD and X-CGD-gp 91^{phox} cells were used to study cell growth and NADPH oxidase activation after treatment with several metal and metalloid compounds (Paper III).

3.3 PRIMARY CELLS

Supplementary studies were performed on the primary human epidermal keratinocytes (HEKa cells) (Paper I).

3.4 MATERIALS

3.4.1 AHR agonists

An indolocarbazole compound, FICZ (Figure 4) instead of the highly toxic compound TCDD (Figure 3) was chosen to assess the biological functions of the AHR in order to understand the intrinsic AHR signaling. TCDD is the prototype for a family of persistent and structurally similar compounds with toxicological and biological effects on human and experimental animals through activation of the AHR signaling (Poland and Knutson, 1982; White and Birnbaum, 2009). The AHR high affinity ligand FICZ was first described in 1987 by Rannug et al. as a photoproduct formed after ultraviolet irradiation of Trp. Among AHR ligands, FICZ exhibits the lowest K_d (70 pM) for AHR binding of all compounds tested so far and it has been suggested as

an endogenous signaling molecule (Rannug et al., 1987). FICZ is an excellent substrate for CYP1A1 (k_{cat}/K_m of $8.1 \times 10^7 \text{ M}^{-1}\text{s}^{-1}$). The catalytic efficiency for FICZ seems to be close to the limit of diffusion and it is a 50 times better substrate compared to the model CYP1A1 substrate 7-ethoxyresorufin (Wincent et al., 2009). However, the pharmacokinetics and pharmacodynamics of FICZ has to be investigated in more details in future studies. AHR activation by TCDD and FICZ was examined in Paper I.

3.4.2 CYP1A1 inhibitors

Different types of chemicals including oxidants, clinical drugs, endogenous and natural substances, biochemical inhibitors, phytochemicals and metals have been shown to inhibit the activity of CYP1A1 (Moorthy et al., 2000; Ueng et al., 2002; Bozcaarmutlu and Arinc, 2004; Oliveira et al., 2004; Baliharova et al., 2005; Chaudhary and Willett, 2006; Mikstacka et al., 2007; Zhang et al., 2008; Sergent et al., 2009a). In most studies, inhibition of 7-ethoxyresorufin-O-deethylase activity (EROD) was chosen to test the inhibitory effects of chemicals on CYP1A1 enzyme activity. Depending on the structure different chemicals can act as a substrate, inhibitor or both substrate/inhibitor of CYP1A1. A study with different types of flavonoids containing methoxy- and hydroxyl-group substitutions suggests that compounds demonstrating high substrate affinity for CYP1A1 might be stronger inhibitors of EROD activity (Androutsopoulos et al., 2011). Shimida et al. suggested that the number and position of hydroxyl and methoxy groups in the structure of flavones are important factors for inhibitory effects of flavones on CYP1A1 (Shimada et al., 2010).

3.4.2.1 Oxidants

H₂O₂ and UVB were chosen to evaluate the inhibitory effects of oxidants on CYP1A1 gene expression and enzyme activity (Paper I). For UVB exposure, HaCaT cells were exposed to UVB light in dishes containing PBS. UVB lamps consisting of six (Philips TL20W/12RS lamp) or two (Philips PL, 36W, UV240 DT, IP20) UV tubes were used to obtain 5-20 mJ/cm² doses of UVB (280-320nm). After irradiation, the PBS was removed and fresh medium was added to the dishes.

3.4.2.2 Polyphenols

AHR activation by the polyphenols, 3'-methoxy-4'-nitroflavone MNF (Paper I), quercetin (QUE), resveratrol (RES) and curcumin (CUR) (Paper II) was investigated (for chemical structure see Figure 8-10). It has been reported that polyphenols are potent inhibitors of CYP1A1 (Chaudhary and Willett, 2006; Mikstacka et al., 2007). Polyphenols have been claimed to exhibit both agonistic and antagonistic effects on AHR signaling (Ciolino et al., 1998a; Ciolino et al., 1998b; Casper et al., 1999; Ciolino et al., 1999; Zhang et al., 2003; Pohl et al., 2006; Sergent et al., 2009b).

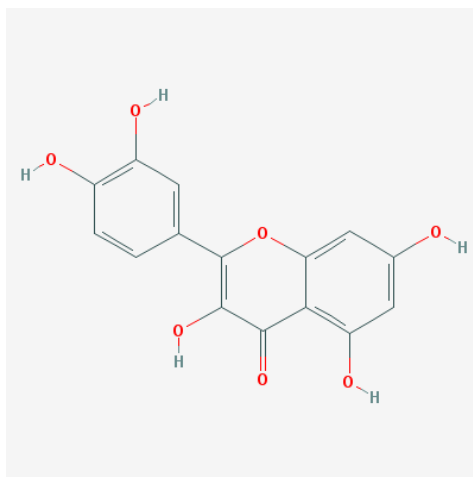


Figure 8. 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one (quercetin)

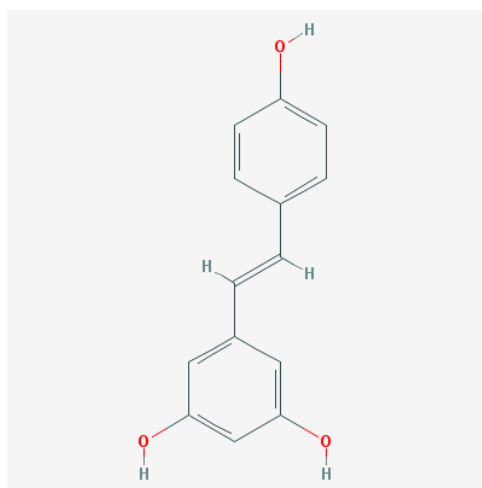


Figure 9. 5-[(E)-2-(4-hydroxyphenyl)ethenyl]benzene-1,3-diol (resveratrol)

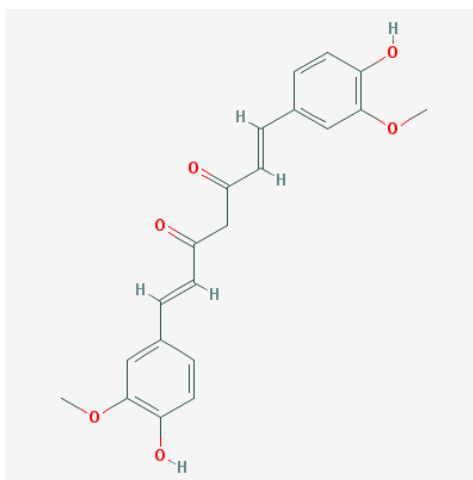


Figure 10. (1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (curcumin)

3.4.2.3 *Metals and metalloids*

AHR activation by metals and metalloids was investigated in Paper III. Arsenic (As), cadmium (Cd), mercury (Hg) and nickel (Ni) were chosen to investigate the mechanism of AHR activation by compounds that are not typical AHR ligands. To investigate the oxidative interaction of metals with AHR signaling was another purpose of this study.

3.5 METHODS

3.5.1 Experimental designs

Dose-response and time course studies were carried out in cells exposed to CYP1A1 inhibitors alone or in combination with FICZ. In all experiments excluding studies with UVB, cells were exposed to fresh medium containing compounds of interest without adding FBS. The treatments were terminated by removing the medium and rinsing the cells with PBS (Paper I, II and III). In UVB experiments, cells were exposed in dishes containing PBS (Paper I). Data were collected and normalized according to the protein content.

In the preconditioning studies, cells were pre-treated with the compounds of interest in the FBS free medium for 5 hours. The pre-treatments were terminated by removing the medium, rinsing the cells with PBS and adding new medium with 10% FBS for 24 hours. After recovery time, second treatments were performed by changing the medium to new medium containing DMSO or FICZ for up to 48 hours (Paper III).

3.5.2 CYP1A1 inhibition assays

Human recombinant CYP1A1 + NADPH-450 reductase supersomes were used to investigate inhibitory effects of chemicals on pure human CYP1A1 by using 7-ethoxyresorufin as a standard substrate (Paper I, II, and III).

3.5.3 Analyses of cellular FICZ content

A HPLC method was used to quantify the levels of FICZ in HaCaT cells exposed to FICZ alone or in combination with CYP1A1 inhibitors. A solid-phase extraction column coupled to a reverse-phase C18 column (Alltech Alltima; 250 mm × 4.6 mm) and a mobile phase consisting of acetonitrile and water were used. FICZ was detected by fluorescence at excitation and emission wavelengths of 390 and 525 nm (Paper I, II and III).

3.5.4 AHR activation assays

Several types of assays have been established to investigate AHR activation by different AHR ligands and CYP1A1 inhibitors. In order to quantify AHR activation at the transcriptional levels either qRT-PCR or luciferase reporter assays were used. CYP1A1 enzyme activity was quantified by the EROD assay (Paper I, II and III).

3.5.4.1 *AHR activation in commercial and purified medium*

In order to find out whether AHR activation by CYP1A1 inhibitors is due to the presence of FICZ in the cell culture media, experiments were performed in a

commercial medium or in a Trp-free medium which was supplemented with fresh and re-crystallized Trp before each use (Paper I, II and III).

3.5.5 Detection of oxidative stress

3.5.5.1 NADPH oxidase activity

Superoxide anion or H₂O₂ produced by NOXs activation was determined by increased lucigenin activity as described previously (Smith et al., 2001). Chemiluminescence was measured on a Genios Pro plate reader after addition of NADPH and lucigenin (Paper III).

3.5.5.2 GSH assay

The level of GSH was determined spectrophotometrically after reaction with 5-5'-dithiobis[2-nitrobenzoic acid] (DTNB) at 405 nm utilizing a Genios Pro plate reader (Paper III).

3.5.5.3 Up-regulated antioxidant genes

Expression of HO-1, CYP1A1 and AHR were determined by RT-qPCR after exposure of HaCaT cells to As. In parallel, adaptive responses to oxidative stress induced by As were determined by predesigned multiple RT-PCR array plates (Stress & Toxicity PathwayFinder PCR Array, PAHS-003Z, SABioscience) consisting of 84 genes involved in 6 biological pathways including oxidative stress, osmotic stress, DNA damage, inflammation, hypoxia and heat shock proteins/unfolded proteins (Paper III).

3.5.6 NOX4 and Nrf2 silencing

A SMART pool siGENOME NOX4 siRNA consisting of four small interfering NOX4 RNA sequences and siRNA against Nrf2 were used to silence NOX4 and Nrf2 pathways. SiRNA complexes were formed in antibiotic- and serum-free DMEM medium, using DharmacoFECT transfection reagents (Paper III).

3.5.7 Cell proliferation assay

The CyQUANT NF cell proliferation assay kit was used to assess cell proliferation in PLB-985 and HaCaT cells treated with different concentrations of metals alone or together with diphenyleneiodonium (DPI). Dye binding to DNA (fluorescence) was measured at excitation/emission wavelengths 492/535 nm (Paper III).

4 AIMS OF PRESENT STUDY

The overall aim of the work included in this thesis was to improve current knowledge regarding the redox regulation of AHR signaling. A particular objective was to study AHR activation in the absence of exogenously added AHR ligands. The project includes characterization of enzymatic breakdown of a metabolizable natural AHR ligand, FICZ.

The specific aims were:

- To explore the effect of some compounds, known to activate AHR without being good ligands for the receptor
- To investigate if the effect of these compounds is caused by the presence of FICZ in the cell culture medium
- To evaluate inhibition of CYP1A1 mediated degradation of the natural AHR ligand FICZ
- To clarify the influence of ROS-generating compounds on AHR signaling pathways

5 RESULTS AND DISCUSSION

5.1 FICZ IS A POTENT CYP1A1 INDUCER IN VITRO AND IN VIVO

Among AHR agonists known to date, FICZ exhibits the highest affinity to bind and activate AHR signaling (Rannug et al., 1987; Fritsche et al., 2007; Jonsson et al., 2009; Wincent et al., 2009). In contrast to TCDD the effects of FICZ on AHR signaling are transient (Wei et al., 1998; Wincent et al., 2009). The ability of FICZ to distribute in the body through systemic circulation and induce CYP1A1 gene expression in peripheral tissues was determined by administration of 10 ng FICZ on one ear of female C57BL/6J mice (Paper I). The results of this experiment showed that FICZ, in spite of its rapid metabolism (Wincent et al., 2009), can be distributed in the body through the systemic circulation and induce CYP1A1 gene expression in several tissues. In our study, the expression of CYP1A1 gene in liver and adipose tissues was transient and a prolonged induction was observed at the site of application. Sustained induction of CYP1A1 at the site of application can be explained by pharmacokinetic parameters of FICZ and presence of FICZ at high concentrations at the site of application. The inhibitory effects of FICZ on its own metabolism by CYP1A1 has been previously reported (Wincent et al., 2009). Therefore, studying pharmacokinetics parameters including absorption, distribution, metabolism and excretion from the body might provide valuable information in order to understand the intrinsic physiology of AHR.

Next, we examined the potential of FICZ as an inducer of CYP1A1 gene expression *in vitro*. The EC₅₀ value (half maximal effective concentration) of FICZ was found to be at picomolar concentration which is lower than the EC₅₀ value earlier described for TCDD (Denison et al., 2002).

Taken together, this study showed that FICZ is a potent inducer of CYP1A1 *in vivo* and *in vitro*. FICZ can distribute in the body through the systemic circulation and induce CYP1A1 in various organs.

5.2 CYP1A1 INHIBITORS TURN INTO AHR ACTIVATORS

5.2.1 Metabolic clearance of FICZ is inhibited by CYP1A1 inhibitors

The potential ability of different groups of chemicals such as H₂O₂, MNF (Paper I), polyphenols (Paper II) and metals (Paper III) to inhibit human recombinant CYP1A1 was evaluated in this thesis. H₂O₂ at 200 µM and MNF at 2.5 µM concentrations gave approximately 90% and 80% inhibition of microsomal CYP1A1 activity, respectively. The half maximal inhibitory concentration (IC₅₀ value) of the other chemicals tested in the experiments was determined by constructing dose-response curves. According to the IC₅₀ values, their potencies to inhibit human recombinant CYP1A1 can be ranked as follows:

QUE (1.2 µM) > CUR (7.3 µM) > RES (11.8 µM)

Hg (0.16 µM) > Cd (1.70 µM) > Ni (14.70 µM) > As (16.02 µM)

Our results are consistent with earlier studies, which have shown that the polyphenols QUE, RES, and CUR are able to inhibit the catalytic activity of CYP1A1 enzyme (Oetari et al., 1996; Ciolino et al., 1998a; Chun et al., 1999; Chaudhary and Willett, 2006). In studies performed with human recombinant CYP1A1 both K_m and V_{max} changed with increasing doses of QUE and RES suggesting a mixed type of inhibition by QUE and RES (Chaudhary and Willett, 2006; Mikstacka et al., 2007). Metals and

metalloids have also earlier been shown to be able to inhibit the activity of CYP1A1 enzyme (Anwar-Mohamed et al., 2009). Metals inhibit the function of CYP1A1 enzymes by interfering with the heme moiety at the catalytic site (Gonzalez, 1988; Vernhet et al., 2003; Kaminsky, 2006). Metals also are able to generate ROS and ROS may oxidize thiol groups in the cysteine residues of proteins (Bogdan, 2001). The inhibition of CYP1A1 by metals seems to be through reversible inhibition. These inhibitors exhibit only dose-dependent inhibition pattern (Hollenberg, 2002). CYP1A1 inhibition by metals can be reversed by addition of GSH or antioxidant (Bozcaarmutlu and Arinc, 2004; Oliveira et al., 2004) suggesting that in addition to interfering with the heme moiety of CYP1A1, generation of ROS is also involved in the inhibition of CYP1A1 by metals.

The effects of CYP1A1 inhibitors on metabolic degradation of FICZ were evaluated by HPLC in HaCaT cells. The cells were treated with FICZ alone or in combination with different doses of H₂O₂ and MNF (Paper I), QUE, RES and CUR (Paper II), As, Cd, Hg and Ni (Paper III) for different lengths of time. All tested compounds were able to inhibit the metabolic degradation of FICZ. Among the three polyphenol compounds tested in this thesis, QUE was the most potent inhibitor of human recombinant CYP1A1 and QUE was also a strong inhibitor of FICZ metabolism in HaCaT cells. Among the metals tested, Hg was the most potent inhibitor of CYP1A1 while no major differences in efficacy of metals to inhibit FICZ degradation were observed. It can be speculated that the pharmacokinetics of FICZ can be influenced by co-treatments with metals. Heavy metals not only interfere with the normal function of metalloproteins such as CYP1A1 but also they are able to disrupt the normal functions of cellular carriers and transporters.

5.2.2 CYP1A1 inhibitors activate AHR indirectly

It has been shown that commercial media contain FICZ and can activate AHR signaling especially if the media had been exposed to light (Oberg et al., 2005).

The ability of AHR agonists such as FICZ and TCDD and several CYP1A1 inhibitors including oxidants, clinical drugs, endogenous and natural substances, and compounds used as biochemical inhibitors (Paper I), polyphenols (Paper II) and metals (Paper III) to activate AHR signaling in the absence of the high affinity ligand FICZ was tested by exposing the cells in a commercial DMEM and a Trp-free DMEM which was supplemented with freshly re-crystallized Trp before each use. The results obtained in the medium lacking FICZ were significantly different from the experiments performed in commercial DMEM.

Next, the background levels of FICZ were quantified in commercial batches of media to find out how much of FICZ is sufficient to activate AHR signaling. The level of FICZ was determined by HPLC and a series of experiments were performed in the purified medium by adding different concentrations of FICZ. The concentration of FICZ in commercial DMEM was found to be in the order of 0.1 pM (Paper I) and this level of FICZ was sufficient to activate AHR signaling as determined in the EROD assay (Paper I).

Based on our studies with different groups of CYP1A1 inhibitors (paper I, II and III), previously established AHR activation by α NF (Wincent et al., 2009), and a wide range of CYP1A1 inhibitors listed in the supplementary table of Paper I (Figure 11) we suggest that an indirect mechanism for AHR activation explains the findings of AHR-induction by compounds that do not fit into the ligand binding pocket of the AHR.

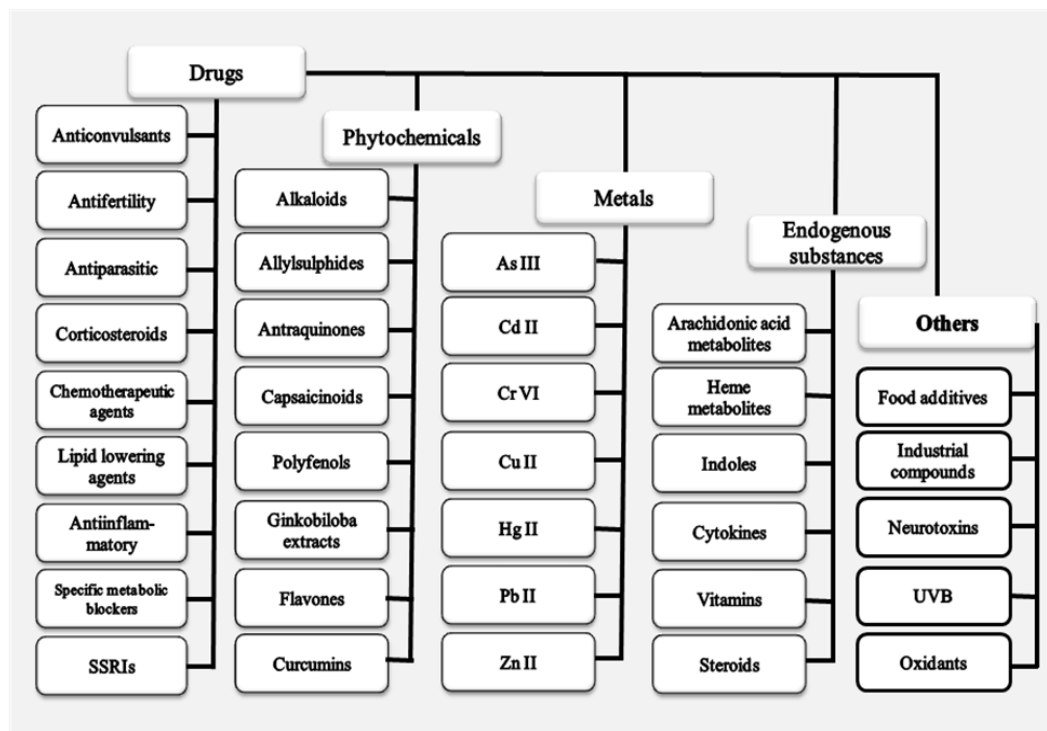


Figure 11. Examples of CYP1A1 inhibitors that are also AHR activators

5.3 OXIDATIVE STRESS MODULATES AHR SIGNALING

5.3.1 Oxidative stress antagonizes AHR activation

Temporal inhibition of CYP1A1 transcription activity at the early time of incubation was seen in HaCaT and HepG2 cells treated with FICZ together with oxidants (Paper I), polyphenols (Paper I and II) and metals followed by an up-regulation of both CYP1A1 transcription and enzyme activity (Paper III). An obvious correlation between AHR activation and intracellular levels of reduced glutathione was suggested by the results presented in Paper III.

Up-regulation of CYP1A1 required relatively reducing conditions as shown by measurement of cellular GSH in parallel with CYP1A1 induction in HepG2 cells. We further confirmed this hypothesis by using a precursor of glutathione, N-Acetyl-L-cysteine (NAC), and a depletory agent of glutathione, buthionine-(S,R)-sulfoximine (BSO) in CYP1A1 induction experiments. In HepG2-XRE-Luc cells co-treated with BSO the attenuation of CYP1A1 transcription activity caused by the metals was potentiated, while NAC treatments efficiently reduced the inhibitory effects.

The effects of polyphenols on CYP1A1 transcription activity were biphasic (Figure 12). FICZ-stimulated AHR transcription was potentiated by low concentrations of polyphenols and inhibited in a dose-dependent manner by high concentrations. It can be speculated that polyphenols at low concentrations act as antioxidants and increase the antioxidant capacity of cells. Phenolic compounds have been suggested to interfere with the oxidation of macromolecules in the cells by donating a hydrogen atom to free radicals. However, under certain conditions polyphenols may act as pro-oxidants (Shahidi and Wanasundara, 1992; Bravo, 1998). It seems plausible that the inhibition of AHR reporter activity that was only observed with μM concentrations was explained by the pro-oxidative effects of the polyphenols.

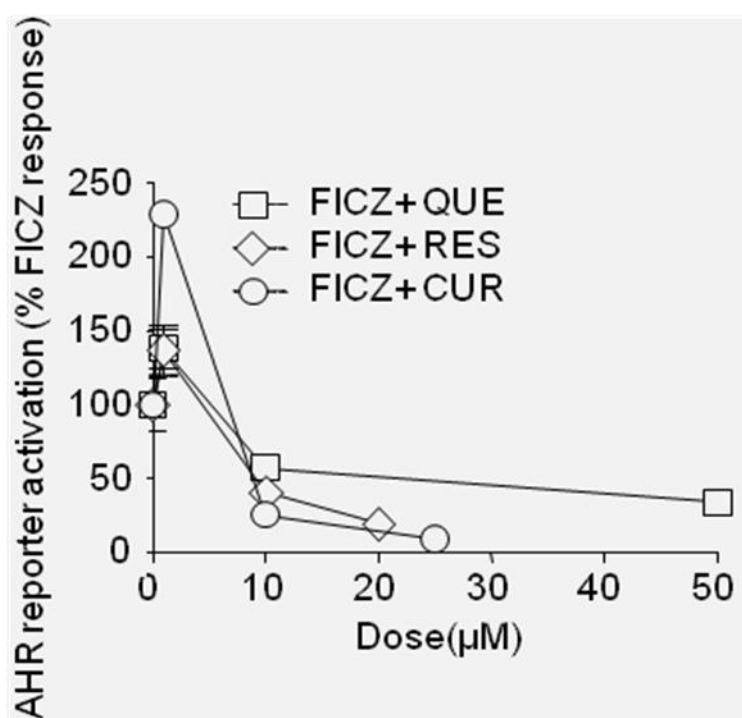


Figure 12. Biphasic effects of polyphenols on the transcription activity of AHR

It is well known that metals induce oxidative stress and cause depletion of GSH by binding to sulfhydryl groups of proteins (Kaminsky, 2006; Jomova and Valko, 2011). Down-regulation of CYP1A1 by oxidants and depletion of GSH has been reported in several studies (Morel and Barouki, 1998; Xu et al., 1998). The AHR protein and the DNA-binding of many transcription factors were earlier shown to be sensitive to oxidative stress (Denison et al., 1987; Pongratz et al., 1992; Droge et al., 1994; Cumming et al., 2004). We hypothesize that the sulfhydryl groups in the chaperone protein HSP90 in the AHR complex and DNA-binding of AHR may be the sensitive targets to oxidative stress.

FICZ, TCDD and some other high affinity AHR ligands function as potent agonists and activators of AHR *in vitro* and *in vivo*. Conversely, α -naphthoflavone, MNF and the most potent AHR inhibitor described so far i.e. CH223191 are compounds used as specific antagonists of AHR (Lu et al., 1995; Gasiewicz et al., 1996; Kim et al., 2006;

Zhao et al., 2010). However, most of the AHR antagonists exert partial agonistic activity at high concentrations as shown by their capacity to compete with a high affinity AHR agonist, in most cases [³H]TCDD, for binding to the receptor and inhibit agonist activated AHR-ARNT complex to bind and activate XRE/DRE-dependent transcription. We have shown that the agonistic properties of some AHR activators to some extent can also be attributed to the presence of the AHR high affinity ligand FICZ in the cell culture media.

CH223191 (Figure 13) has not been reported to activate AHR and is therefore suggested to be a pure and potent AHR antagonist. CH223191 also inhibited the AHR-activation in HepG2-XRE-Luc cells in our studies (Figure 14). However, the antagonistic effects of CH223191 on FICZ stimulated AHR activation could, to some extent, be reversed by addition of the potent antioxidant NAC (Figure 14), which suggest that CH223191 is an oxidant which like other oxidants can lower the levels of GSH and thereby cause a temporal inhibition of AHR regulated responses. This result suggests that like the commonly used AHR antagonists α -naphthoflavone and MNF, also CH223191 can work as an oxidant and inhibit AHR signaling by this mechanism. The sensitivity of AHR complex to oxidants has been further confirmed by results showing the ability of the reducing agent dithiothreitol to restore the displacement of [³H]TCDD from AHR complex by the sulfhydryl-modifying reagent, Hg (Denison et al., 1987).

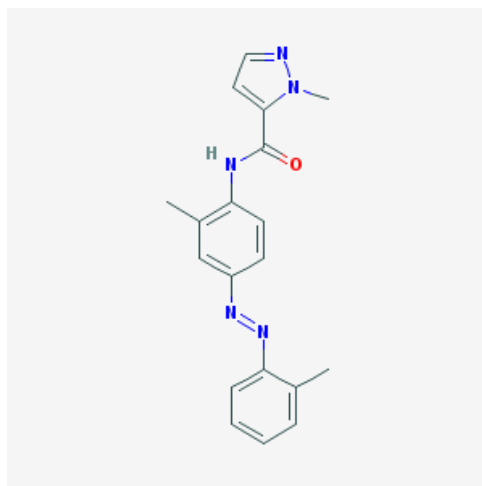


Figure 13. Structure of the potent AHR antagonist 2-methyl-N-[2-methyl-4-[(2-methylphenyl)diazenyl]phenyl]pyrazole-3-carboxamide (CH223191)

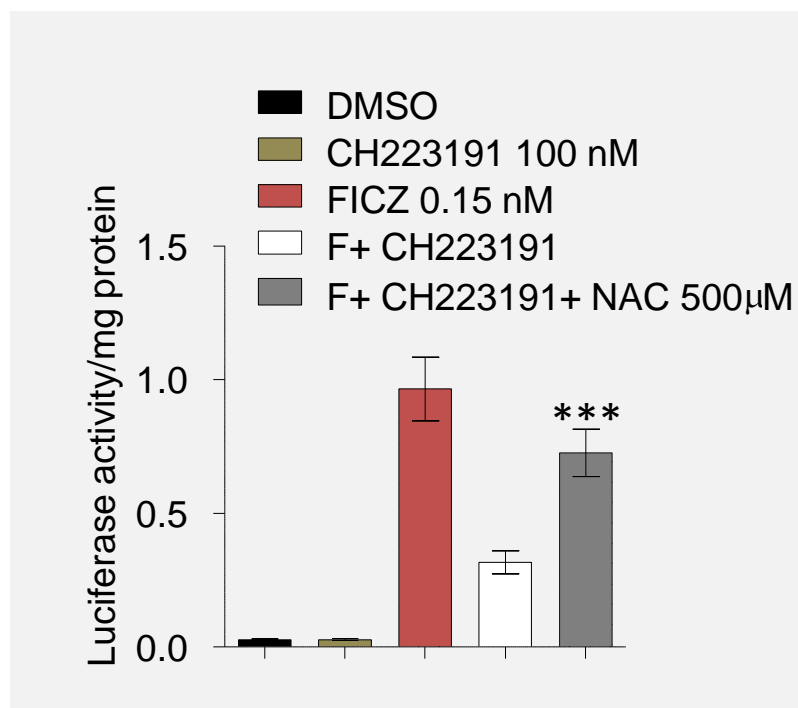


Figure 14. Effects of NAC on AHR inhibition by CH223191

5.3.2 Oxidative stress up-regulates antioxidant genes

In response to As treatment we observed a substantial (1000-fold) up-regulation of HO-1 and more than 2 fold induction of 16 genes related to oxidative stress, osmotic stress, DNA damage, inflammation, hypoxia and heat shock proteins/unfolded proteins (Paper III). In parallel with up-regulation of the modifier subunit and the catalytic subunit of GCL we observed that the level of GSH increased during the time of incubation. The common mechanisms for up-regulation of antioxidant genes are mainly mediated by activation of redox-sensitive transcription factors. Low intensity of ROS activates the Keap1/Nrf2 pathway system to up-regulate genes encoding antioxidant enzymes such as the GCL. Intermediate intensity of ROS activates NF- κ B and MAP-kinases pathways to induce antioxidant genes and inflammation responses and high levels of ROS lead to apoptosis and necrosis which is mainly mediated by mitochondria-dependent death cascades (Gloire et al., 2006; Lushchak, 2011).

5.3.3 Preconditioning with oxidants leads to super-induction of CYP1A1

Preconditioning with H₂O₂ or metals significantly elevated the level of GSH in HepG2-XRE-Luc cells. In accordance with these results the AHR transcription machinery induction by endogenous ligand FICZ was very effective, when cells were in a highly reduced form, (Paper III).

The steady-state level of CYPs mRNA is a result of mRNA synthesis and mRNA degradation. Among CYPs mRNAs, CYP1A1 possesses a short half-life (2.4-4.4 h) (Lekas et al., 2000; Suzuki and Nohara, 2007). After translation of CYPs mRNA to protein, the level of enzymes can be regulated by post-translational modifications (Aguiar et al., 2005; Oesch-Bartlomowicz and Oesch, 2005). This suggests that ROS-mediated increases of antioxidant enzymes change the redox levels of the cells so that

the AHR transcriptional machinery or the half-life AHR-regulated RNAs or proteins are prolonged. Accordingly, overexpression of antioxidant enzymes such as superoxide dismutase or catalase in mouse aortic endothelial cells (MAECs) has been shown to increase the induction of CYP1A1 mRNA and protein after benzo[a]pyrene treatment (Wang et al., 2009; Tang et al., 2010).

Since the AHR protein has a considerably short half-life, stabilization of the protein could be another explanation for super-induction of CYP1A1.

5.4 NADPH OXIDASE HAS A CENTRAL ROLE IN REGULATING CELL GROWTH AND ARYL HYDROCARBON RECEPTOR SIGNALING

5.4.1 NOXs stimulate cell growth

NOXs can be activated by metals as documented in our studies (Paper III) and with other stimuli (Chou et al., 2004; Rockwell et al., 2004; Cuypers et al., 2010; Furieri et al., 2011). Superoxide anion or H₂O₂ produced by NOX enzymes are known to play a crucial role in stimulation of cell proliferation (Sturrock et al., 2006; Chan et al., 2009). We observed that administration of a NOX inhibitor or using a mutant X-CGD cell line lacking a functional gp91^{phox} inhibited the stimulation of cell growth that was caused by exposure to low levels of metals. This clearly showed that NOX-derived superoxide anion or H₂O₂ was responsible for the growth stimulation by As and the other metals. Stimulation of cell growth at low concentrations and inhibition at high concentrations of metals as observed in this study has also been reported in other studies with inorganic chemicals (Calabrese and Baldwin, 2003).

5.4.2 NOXs have biphasic effects on AHR signaling pathway

The mechanism of down-regulation of CYP1A1 by oxidants is not well known. Another approach of this study was therefore to evaluate the interference of NOX activation on the AHR signaling pathway. It has been reported that several NOX activators such as H₂O₂, oxidized low density lipoproteins (oxLDL), TGF α , and β , IL-1 α and β , EGF, TNF α and INF γ are repressors of CYP1A1 (Hohne et al., 1990; Ballou et al., 1992; Abdel-Razzak et al., 1993; Barker et al., 1994; Muntane-Relat et al., 1995; Muller et al., 2000; Thum and Borlak, 2004; Hollingshead et al., 2008). All metals tested in this study were able to activate NOXs and inhibit the activity of CYP1A1 (Paper III). The inhibitory effects of metals on FICZ-stimulated AHR activation were transient. We found that the temporal inhibition of CYP1A1 was attenuated by siRNA against NOX4 or administration of DPI a pharmacological inhibitor of NOXs, suggesting an important role for NOX-derived ROS in down-regulation of CYP1A1.

Temporal inhibition of CYP1A1 was followed by a prolonged and sustained induction of CYP1A1 at later incubation times. This late induction of CYP1A1 was abolished by silencing of the Nrf2 pathway (Paper III) indicating that Nrf2 gives up-regulation of antioxidant genes and increasing the level of GSH and thereby increasing the efficacy of the AHR as was seen in the cases of pre-conditioning with metals and H₂O₂. NOX-derived ROS or H₂O₂ seems to be important signals for activation of the keap1/Nrf2 complex and up-regulation of antioxidants and phase II genes through the antioxidant response element (Chan et al., 2009; Brewer et al., 2011; Jiang et al., 2011; Schroder et

al., 2012). Taken together, oxidative stress can negatively and positively modulate the AHR signaling pathway. Therefore, FICZ/AHR/CYP1 auto-regulation is highly influenced by oxidative stress and this might have potentially harmful consequences.

6 CONCLUDING REMARKS AND FUTURE PERSPECTIVES

The inhibition of metabolic turnover of the endogenous ligand FICZ was found to be a mechanism for activation of AHR signaling by several oxidants, metals and phytochemicals. This finding explains how compounds can activate AHR indirectly. In many studies the AHR-activating capacity of natural and synthetic molecules has been established in the presence of the high affinity AHR ligand FICZ (commercial media which contain FICZ) and it can be assumed that the background levels of FICZ have contributed to the results of such experiments.

We found that redox-active compounds regulate the catalytic turnover of the endogenous AHR activator FICZ by inhibiting and inducing AHR-mediated transcription of CYP1A1 and the catalytic efficiency of the CYP1A1 protein. We can conclude that the FICZ/AHR/CYP1 auto-regulation is highly influenced by changes in the redox status of cells. The current *in vitro* studies thus can explain why many oxidants behave both as AHR antagonists and agonists. To further confirm these findings, additional *in vivo* experiments and mechanistic studies are needed.

We also found that the induction of AHR target genes such as CYP1A1 by the AHR endogenous ligand FICZ is transient and suggest that prolonged induction of AHR activity by CYP1A1 inhibitors might have potentially harmful consequences and lead to developmental and physiological disorders.

Most often, humans are exposed to a mixture of toxic chemicals. Co-exposure to synthetic chemicals and heavy metals may enhance the risk of cancer as well as non-cancerous diseases in human. Due to the persistence of heavy metals, PAHs and other AHR activators in the environment, drinking water and food supplies interaction of these compounds with the endogenously activated AHR signaling pathway can be of considerable importance.

7 ACKNOWLEDGEMENTS

This work was performed at the Institute of Environmental Medicine, Karolinska Institutet and Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University. I wish to express my sincere gratitude to everyone who contributed throughout this thesis, and especially to:

My main supervisor Agneta Rannug, first of all for giving me the opportunity to join her group and all support throughout the PhD studies. I am so much indebted to her for introducing me to a new interesting field of research (AHR signaling and its endogenous ligand, FICZ). Thank you so much for all the great scientific discussions between us during this period. I am thankful for the amount of time you spent with me and the other PhD students. I would like to thank you for creating a very excellent scientific environment in our group and sharing your knowledge, opinion and your experience every time with us. I am thankful for critically reading my thesis and her valuable comments and expert editorial assistance.

Professor Ulf Rannug and Ralf Morgenstern, for all that you have taught me and your help and co-supervision to conduct my PhD education. I am thankful for critically reading my papers and thesis and your invaluable comments. Ulf many thanks for teaching me analytical techniques and HPLC.

I would like to thank Gunnar Johanson, head of the unit of Work Environment Toxicology for creating an excellent scientific environment at the division and for all the study visits and social activities during four years.

I wish to also thank all my collaborators for fruitful collaboration on my thesis: Johanna Bengtsson, Tomas Alsberg, Sandra Luecke, Linda Vikström Bergander and Anna Smirnova. I wish to especially mention Emma Wincent, for great discussions between us about science and social activities. I am very thankful that you shared your profound knowledge and ideas with me and for contributing with papers and your excellent comments on my second paper.

My sincere gratitude also goes to my mentors, Reza Mohammadi and Hossein Niknahad and my former supervisor Mahmoud Ghazi-Khansari for your invaluable guidance during my studying.

I would like to thank all my past and present colleagues at Unit of Work Environment Toxicology and IMM: Anders Iregren, Anna-Karin Mörk, Anna-Karin Alexandrie, Birgitta Lindell, Fereshteh Kalantari, Joakim Ringblom, Johan Montelius, Kristin Larsson, Kristin Stamyr. Mei Hong, Lena Ernstgård, Linda Schenk, Mattias Rauma, Mattias Öberg, Mia Johansson, Mishra Dwivedi, Stepanie Juran, Tao Liu, Ulrika Carlander, Johnny Lorentzeh, Monica Nordberg, Irina Svechnikova, Jill Järnberg, Cecilia Wallin, Barbara Julia Arroyo, Dashti Sinjawi, Ali Soroush, Omid Beiki, Gholamreza Abdoli, Mohammad Mohammadi, Mohsen Besharatpour, Imran Ali, Bahareh Rasouli, Zahra Golabkesh, Maral Adel Fahmideh, Siraz Shaik and Huzan Hussien.

I would like to thank Ian Jarvis and Mohammad Amin Tabatabaiefar for reading the thesis and language advice.

This work was supported by the Swedish Research Council for Environment, Agricultural Science and Spatial Planning (FORMAS). I would like to acknowledge them for their support and also to thank the Scholarship office at the Ministry of Health and Medical Education of IRAN and Shiraz University of Medical Sciences for awarding me a full scholarship to pursue my PhD degree in toxicology.

I would like to thank Dr. Abdollahi and Dr. Meshkatoddini and all people at the Scientific Counselor of Iran in the Schengen Area.

I would like to thank Bengt Sjögren for inviting me to play football with Mundialistas AB and his kind help and support during my studying.

Finally, I would like to express my deepest gratitude and love towards my family: my parents, my brothers and sisters for all your support and caring during my life. I would never have been here without your kind support, encouragement and guidance. At last but not least, the kind supports endowed by my brothers and sisters especially, Morteza Moslem and Mahin, and finally my uncle, Najaf, is highly appreciated.

8 REFERENCES

- Abdel-Razzak, Z., Loyer, P., Fautrel, A., Gautier, J.C., Corcos, L., Turlin, B., Beaune, P. and Guillouzo, A. (1993) Cytokines down-regulate expression of major cytochrome P-450 enzymes in adult human hepatocytes in primary culture. *Mol Pharmacol* 44, 707-15.
- Adachi, J., Mori, Y., Matsui, S., Takigami, H., Fujino, J., Kitagawa, H., Miller, C.A., 3rd, Kato, T., Saeki, K. and Matsuda, T. (2001) Indirubin and indigo are potent aryl hydrocarbon receptor ligands present in human urine. *J Biol Chem* 276, 31475-8.
- Aguiar, M., Masse, R. and Gibbs, B.F. (2005) Regulation of cytochrome P450 by posttranslational modification. *Drug Metab Rev* 37, 379-404.
- Akerboom, T.P., Bilzer, M. and Sies, H. (1982) The relationship of biliary glutathione disulfide efflux and intracellular glutathione disulfide content in perfused rat liver. *J Biol Chem* 257, 4248-52.
- Ambasta, R.K., Kumar, P., Griendling, K.K., Schmidt, H.H., Busse, R. and Brandes, R.P. (2004) Direct interaction of the novel Nox proteins with p22phox is required for the formation of a functionally active NADPH oxidase. *J Biol Chem* 279, 45935-41.
- Androutsopoulos, V.P., Papakyriakou, A., Vourloumis, D. and Spandidos, D.A. (2011) Comparative CYP1A1 and CYP1B1 substrate and inhibitor profile of dietary flavonoids. *Bioorg Med Chem* 19, 2842-9.
- Anwar-Mohamed, A., Elbekai, R.H. and El-Kadi, A.O. (2009) Regulation of CYP1A1 by heavy metals and consequences for drug metabolism. *Expert Opin Drug Metab Toxicol* 5, 501-21.
- Baba, T., Mimura, J., Gradin, K., Kuroiwa, A., Watanabe, T., Matsuda, Y., Inazawa, J., Sogawa, K. and Fujii-Kuriyama, Y. (2001) Structure and expression of the Ah receptor repressor gene. *J Biol Chem* 276, 33101-10.
- Baba, T., Mimura, J., Nakamura, N., Harada, N., Yamamoto, M., Morohashi, K. and Fujii-Kuriyama, Y. (2005) Intrinsic function of the aryl hydrocarbon (dioxin) receptor as a key factor in female reproduction. *Mol Cell Biol* 25, 10040-51.
- Baliyarova, V., Velik, J., Fimanova, K., Lamka, J., Szotakova, B., Savlik, M. and Skalova, L. (2005) Inhibitory effect of albendazole and its metabolites on cytochromes P450 activities in rat and mouflon in vitro. *Pharmacol Rep* 57, 97-106.
- Ballou, L.R., Chao, C.P., Holness, M.A., Barker, S.C. and Raghow, R. (1992) Interleukin-1-mediated PGE2 production and sphingomyelin metabolism. Evidence for the regulation of cyclooxygenase gene expression by sphingosine and ceramide. *J Biol Chem* 267, 20044-50.
- Barker, C.W., Fagan, J.B. and Pasco, D.S. (1994) Down-regulation of P4501A1 and P4501A2 mRNA expression in isolated hepatocytes by oxidative stress. *J Biol Chem* 269, 3985-90.
- Barouki, R., Coumoul, X. and Fernandez-Salguero, P.M. (2007) The aryl hydrocarbon receptor, more than a xenobiotic-interacting protein. *FEBS Lett* 581, 3608-15.
- Beedanagari, S.R., Taylor, R.T., Bui, P., Wang, F., Nickerson, D.W. and Hankinson, O. (2010) Role of epigenetic mechanisms in differential regulation of the dioxin-inducible human CYP1A1 and CYP1B1 genes. *Mol Pharmacol* 78, 608-16.
- Bell, D.R. and Poland, A. (2000) Binding of aryl hydrocarbon receptor (AhR) to AhR-interacting protein. The role of hsp90. *J Biol Chem* 275, 36407-14.
- Benedict, J.C., Lin, T.M., Loeffler, I.K., Peterson, R.E. and Flaws, J.A. (2000) Physiological role of the aryl hydrocarbon receptor in mouse ovary development. *Toxicol Sci* 56, 382-8.
- Berg, P. and Pongratz, I. (2001) Differential usage of nuclear export sequences regulates intracellular localization of the dioxin (aryl hydrocarbon) receptor. *J Biol Chem* 276, 43231-8.
- Bjeldanes, L.F., Kim, J.Y., Grose, K.R., Bartholomew, J.C. and Bradfield, C.A. (1991) Aromatic hydrocarbon responsiveness-receptor agonists generated from indole-

- 3-carbinol in vitro and in vivo: comparisons with 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Proc Natl Acad Sci U S A* 88, 9543-7.
- Bock, K.W. (2012) The human Ah receptor: hints from dioxin toxicities to deregulated target genes and physiologic functions. *Biol Chem*. Paper in press.
- Bogdan, C. (2001) Nitric oxide and the regulation of gene expression. *Trends Cell Biol* 11, 66-75.
- Boitano, A.E., Wang, J., Romeo, R., Bouchez, L.C., Parker, A.E., Sutton, S.E., Walker, J.R., Flaveny, C.A., Perdew, G.H., Denison, M.S., Schultz, P.G. and Cooke, M.P. (2010) Aryl hydrocarbon receptor antagonists promote the expansion of human hematopoietic stem cells. *Science* 329, 1345-8.
- Bozcaarmutlu, A. and Arinc, E. (2004) Inhibitory effects of divalent metal ions on liver microsomal 7-ethoxyresorufin O-deethylase (EROD) activity of leaping mullet. *Mar Environ Res* 58, 521-4.
- Bravo, L. (1998) Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutr Rev* 56, 317-33.
- Brewer, A.C., Murray, T.V., Arno, M., Zhang, M., Anilkumar, N.P., Mann, G.E. and Shah, A.M. (2011) Nox4 regulates Nrf2 and glutathione redox in cardiomyocytes in vivo. *Free Radic Biol Med* 51, 205-15.
- Brown, G.C. and Borutaite, V. (2012) There is no evidence that mitochondria are the main source of reactive oxygen species in mammalian cells. *Mitochondrion* 12, 1-4.
- Calabrese, E.J. and Baldwin, L.A. (2003) Inorganics and hormesis. *Crit Rev Toxicol* 33, 215-304.
- Carver, L.A. and Bradfield, C.A. (1997) Ligand-dependent interaction of the aryl hydrocarbon receptor with a novel immunophilin homolog in vivo. *J Biol Chem* 272, 11452-6.
- Carver, L.A., Jackiw, V. and Bradfield, C.A. (1994) The 90-kDa heat shock protein is essential for Ah receptor signaling in a yeast expression system. *J Biol Chem* 269, 30109-12.
- Carver, L.A., LaPres, J.J., Jain, S., Dunham, E.E. and Bradfield, C.A. (1998) Characterization of the Ah receptor-associated protein, ARA9. *J Biol Chem* 273, 33580-7.
- Casado, F.L., Singh, K.P. and Gasiewicz, T.A. (2010) The aryl hydrocarbon receptor: regulation of hematopoiesis and involvement in the progression of blood diseases. *Blood Cells Mol Dis* 44, 199-206.
- Casper, R.F., Quesne, M., Rogers, I.M., Shiota, T., Jolivet, A., Milgrom, E. and Savouret, J.F. (1999) Resveratrol has antagonist activity on the aryl hydrocarbon receptor: implications for prevention of dioxin toxicity. *Mol Pharmacol* 56, 784-90.
- Chan, E.C., Jiang, F., Peshavariya, H.M. and Dusting, G.J. (2009) Regulation of cell proliferation by NADPH oxidase-mediated signaling: potential roles in tissue repair, regenerative medicine and tissue engineering. *Pharmacol Ther* 122, 97-108.
- Chang, C.Y. and Puga, A. (1998) Constitutive activation of the aromatic hydrocarbon receptor. *Mol Cell Biol* 18, 525-35.
- Chaudhary, A. and Willett, K.L. (2006) Inhibition of human cytochrome CYP 1 enzymes by flavonoids of St. John's wort. *Toxicology* 217, 194-205.
- Chen, Y.H., Riby, J., Srivastava, P., Bartholomew, J., Denison, M. and Bjeldanes, L. (1995) Regulation of CYP1A1 by indolo[3,2-b]carbazole in murine hepatoma cells. *J Biol Chem* 270, 22548-55.
- Cheng, G., Cao, Z., Xu, X., van Meir, E.G. and Lambeth, J.D. (2001) Homologs of gp91phox: cloning and tissue expression of Nox3, Nox4, and Nox5. *Gene* 269, 131-40.
- Chiaro, C.R., Patel, R.D., Marcus, C.B. and Perdew, G.H. (2007) Evidence for an aryl hydrocarbon receptor-mediated cytochrome p450 autoregulatory pathway. *Mol Pharmacol* 72, 1369-79.
- Chou, W.C., Jie, C., Kenedy, A.A., Jones, R.J., Trush, M.A. and Dang, C.V. (2004) Role of NADPH oxidase in arsenic-induced reactive oxygen species formation and cytotoxicity in myeloid leukemia cells. *Proc Natl Acad Sci U S A* 101, 4578-83.

- Chun, Y.J., Kim, M.Y. and Guengerich, F.P. (1999) Resveratrol is a selective human cytochrome P450 1A1 inhibitor. *Biochem Biophys Res Commun* 262, 20-4.
- Ciolino, H.P., Daschner, P.J., Wang, T.T. and Yeh, G.C. (1998a) Effect of curcumin on the aryl hydrocarbon receptor and cytochrome P450 1A1 in MCF-7 human breast carcinoma cells. *Biochem Pharmacol* 56, 197-206.
- Ciolino, H.P., Daschner, P.J. and Yeh, G.C. (1998b) Resveratrol inhibits transcription of CYP1A1 in vitro by preventing activation of the aryl hydrocarbon receptor. *Cancer Res* 58, 5707-12.
- Ciolino, H.P., Daschner, P.J. and Yeh, G.C. (1999) Dietary flavonols quercetin and kaempferol are ligands of the aryl hydrocarbon receptor that affect CYP1A1 transcription differentially. *Biochem J* 340 (Pt 3), 715-22.
- Cox, M.B. and Miller, C.A., 3rd. (2002) The p23 co-chaperone facilitates dioxin receptor signaling in a yeast model system. *Toxicol Lett* 129, 13-21.
- Crawford, R.B., Holsapple, M.P. and Kaminski, N.E. (1997) Leukocyte activation induces aryl hydrocarbon receptor up-regulation, DNA binding, and increased Cyp1a1 expression in the absence of exogenous ligand. *Mol Pharmacol* 52, 921-7.
- Cumming, R.C., Andon, N.L., Haynes, P.A., Park, M., Fischer, W.H. and Schubert, D. (2004) Protein disulfide bond formation in the cytoplasm during oxidative stress. *J Biol Chem* 279, 21749-58.
- Cuypers, A., Plusquin, M., Remans, T., Jozefczak, M., Keunen, E., Gielen, H., Opdenakker, K., Nair, A.R., Munters, E., Artois, T.J., Nawrot, T., Vangronsveld, J. and Smeets, K. (2010) Cadmium stress: an oxidative challenge. *Biometals* 23, 927-40.
- Davarinos, N.A. and Pollenz, R.S. (1999) Aryl hydrocarbon receptor imported into the nucleus following ligand binding is rapidly degraded via the cytoplasmic proteasome following nuclear export. *J Biol Chem* 274, 28708-15.
- De Deken, X., Wang, D., Dumont, J.E. and Miot, F. (2002) Characterization of ThOX proteins as components of the thyroid H₂O₂-generating system. *Exp Cell Res* 273, 187-96.
- DeLeve, L.D. and Kaplowitz, N. (1991) Glutathione metabolism and its role in hepatotoxicity. *Pharmacol Ther* 52, 287-305.
- Denison, M.S. and Nagy, S.R. (2003) Activation of the aryl hydrocarbon receptor by structurally diverse exogenous and endogenous chemicals. *Annu Rev Pharmacol Toxicol* 43, 309-34.
- Denison, M.S., Pandini, A., Nagy, S.R., Baldwin, E.P. and Bonati, L. (2002) Ligand binding and activation of the Ah receptor. *Chem Biol Interact* 141, 3-24.
- Denison, M.S., Soshilov, A.A., He, G., DeGroot, D.E. and Zhao, B. (2011) Exactly the same but different: promiscuity and diversity in the molecular mechanisms of action of the aryl hydrocarbon (dioxin) receptor. *Toxicol Sci* 124, 1-22.
- Denison, M.S., Vella, L.M. and Okey, A.B. (1987) Structure and function of the Ah receptor: sulfhydryl groups required for binding of 2,3,7,8-tetrachlorodibenzo-p-dioxin to cytosolic receptor from rodent livers. *Arch Biochem Biophys* 252, 388-95.
- Diani-Moore, S., Labitzke, E., Brown, R., Garvin, A., Wong, L. and Rifkind, A.B. (2006) Sunlight generates multiple tryptophan photoproducts eliciting high efficacy CYP1A induction in chick hepatocytes and in vivo. *Toxicol Sci* 90, 96-110.
- Ding, C., Kume, A., Bjorgvinsdottir, H., Hawley, R.G., Pech, N. and Dinauer, M.C. (1996) High-level reconstitution of respiratory burst activity in a human X-linked chronic granulomatous disease (X-CGD) cell line and correction of murine X-CGD bone marrow cells by retroviral-mediated gene transfer of human gp91phox. *Blood* 88, 1834-40.
- Dinkova-Kostova, A.T., Holtzclaw, W.D., Cole, R.N., Itoh, K., Wakabayashi, N., Katoh, Y., Yamamoto, M. and Talalay, P. (2002) Direct evidence that sulfhydryl groups of Keap1 are the sensors regulating induction of phase 2 enzymes that protect against carcinogens and oxidants. *Proc Natl Acad Sci U S A* 99, 11908-13.

- Dolwick, K.M., Swanson, H.I. and Bradfield, C.A. (1993) In vitro analysis of Ah receptor domains involved in ligand-activated DNA recognition. *Proc Natl Acad Sci U S A* 90, 8566-70.
- Droge, W. (2002) Free radicals in the physiological control of cell function. *Physiol Rev* 82, 47-95.
- Droge, W., Schulze-Osthoff, K., Mihm, S., Galter, D., Schenk, H., Eck, H.P., Roth, S. and Gmunder, H. (1994) Functions of glutathione and glutathione disulfide in immunology and immunopathology. *FASEB J* 8, 1131-8.
- Elizondo, G., Fernandez-Salguero, P., Sheikh, M.S., Kim, G.Y., Fornace, A.J., Lee, K.S. and Gonzalez, F.J. (2000) Altered cell cycle control at the G(2)/M phases in aryl hydrocarbon receptor-null embryo fibroblast. *Mol Pharmacol* 57, 1056-63.
- Esser, C., Rannug, A. and Stockinger, B. (2009) The aryl hydrocarbon receptor in immunity. *Trends Immunol* 30, 447-54.
- Fernandez-Salguero, P., Pineau, T., Hilbert, D.M., McPhail, T., Lee, S.S., Kimura, S., Nebert, D.W., Rudikoff, S., Ward, J.M. and Gonzalez, F.J. (1995) Immune system impairment and hepatic fibrosis in mice lacking the dioxin-binding Ah receptor. *Science* 268, 722-6.
- Fernandez-Salguero, P.M., Hilbert, D.M., Rudikoff, S., Ward, J.M. and Gonzalez, F.J. (1996) Aryl-hydrocarbon receptor-deficient mice are resistant to 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced toxicity. *Toxicol Appl Pharmacol* 140, 173-9.
- Fisher, J.M., Wu, L., Denison, M.S. and Whitlock, J.P., Jr. (1990) Organization and function of a dioxin-responsive enhancer. *J Biol Chem* 265, 9676-81.
- Franklin, C.C., Backos, D.S., Mohar, I., White, C.C., Forman, H.J. and Kavanagh, T.J. (2009) Structure, function, and post-translational regulation of the catalytic and modifier subunits of glutamate cysteine ligase. *Mol Aspects Med* 30, 86-98.
- Fritsche, E., Schafer, C., Calles, C., Bernsmann, T., Bernshausen, T., Wurm, M., Hubenthal, U., Cline, J.E., Hajimiragha, H., Schroeder, P., Klotz, L.O., Rannug, A., Furst, P., Hanenberg, H., Abel, J. and Krutmann, J. (2007) Lightning up the UV response by identification of the arylhydrocarbon receptor as a cytoplasmatic target for ultraviolet B radiation. *Proc Natl Acad Sci U S A* 104, 8851-6.
- Fujii-Kuriyama, Y. and Kawajiri, K. (2010) Molecular mechanisms of the physiological functions of the aryl hydrocarbon (dioxin) receptor, a multifunctional regulator that senses and responds to environmental stimuli. *Proc Jpn Acad Ser B Phys Biol Sci* 86, 40-53.
- Fujii-Kuriyama, Y. and Mimura, J. (2005) Molecular mechanisms of AhR functions in the regulation of cytochrome P450 genes. *Biochem Biophys Res Commun* 338, 311-7.
- Fukunaga, B.N. and Hankinson, O. (1996) Identification of a novel domain in the aryl hydrocarbon receptor required for DNA binding. *J Biol Chem* 271, 3743-9.
- Furieri, L.B., Galan, M., Avendano, M.S., Garcia-Redondo, A.B., Aguado, A., Martinez, S., Cachafeiro, V., Bartolome, M.V., Alonso, M.J., Vassallo, D.V. and Salaices, M. (2011) Endothelial dysfunction of rat coronary arteries after exposure to low concentrations of mercury is dependent on reactive oxygen species. *Br J Pharmacol* 162, 1819-31.
- Furness, S.G., Lees, M.J. and Whitelaw, M.L. (2007) The dioxin (aryl hydrocarbon) receptor as a model for adaptive responses of bHLH/PAS transcription factors. *FEBS Lett* 581, 3616-25.
- Gasiewicz, A.T. and Henry, C.E. (2012) History of research on the AHR. In: R. Pohjanvirta (Ed) *The AH Receptor in Biology and Toxicology*. John Wiley & Sons, Inc., Hoboken, New Jersey, p. 3-32.
- Gasiewicz, T.A., Kende, A.S., Rucci, G., Whitney, B. and Willey, J.J. (1996) Analysis of structural requirements for Ah receptor antagonist activity: ellipticines, flavones, and related compounds. *Biochem Pharmacol* 52, 1787-803.
- Gillner, M., Bergman, J., Cambillau, C. and Gustafsson, J.A. (1989) Interactions of rutaecarpine alkaloids with specific binding sites for 2,3,7,8-tetrachlorodibenzo-p-dioxin in rat liver. *Carcinogenesis* 10, 651-4.

- Gloire, G., Legrand-Poels, S. and Piette, J. (2006) NF-kappaB activation by reactive oxygen species: fifteen years later. *Biochem Pharmacol* 72, 1493-505.
- Gonzalez, F.J. (1988) The molecular biology of cytochrome P450s. *Pharmacol Rev* 40, 243-88.
- Gradin, K., Whitelaw, M.L., Toftgard, R., Poellinger, L. and Berghard, A. (1994) A tyrosine kinase-dependent pathway regulates ligand-dependent activation of the dioxin receptor in human keratinocytes. *J Biol Chem* 269, 23800-7.
- Groeger, G., Quiney, C. and Cotter, T.G. (2009) Hydrogen peroxide as a cell-survival signaling molecule. *Antioxid Redox Signal* 11, 2655-71.
- Gu, Y.Z., Hogenesch, J.B. and Bradfield, C.A. (2000) The PAS superfamily: sensors of environmental and developmental signals. *Annu Rev Pharmacol Toxicol* 40, 519-61.
- Haarmann-Stemmann, T. and Abel, J. (2006) The arylhydrocarbon receptor repressor (AhRR): structure, expression, and function. *Biol Chem* 387, 1195-9.
- Haarmann-Stemmann, T., Bothe, H. and Abel, J. (2009) Growth factors, cytokines and their receptors as downstream targets of arylhydrocarbon receptor (AhR) signaling pathways. *Biochem Pharmacol* 77, 508-20.
- Haarmann-Stemmann, T., Bothe, H., Kohli, A., Sydlik, U., Abel, J. and Fritsche, E. (2007) Analysis of the transcriptional regulation and molecular function of the aryl hydrocarbon receptor repressor in human cell lines. *Drug Metab Dispos* 35, 2262-9.
- Habano, W., Gamo, T., Sugai, T., Otsuka, K., Wakabayashi, G. and Ozawa, S. (2009) CYP1B1, but not CYP1A1, is downregulated by promoter methylation in colorectal cancers. *Int J Oncol* 34, 1085-91.
- Hahn, M.E., Allan, L.L. and Sherr, D.H. (2009) Regulation of constitutive and inducible AHR signaling: complex interactions involving the AHR repressor. *Biochem Pharmacol* 77, 485-97.
- Halliwell, B. (2007) Biochemistry of oxidative stress. *Biochem Soc Trans* 35, 1147-50.
- Halliwell, B. and Cross, C.E. (1994) Oxygen-derived species: their relation to human disease and environmental stress. *Environ Health Perspect* 102 Suppl 10, 5-12.
- Han, X., Berardi, P. and Riabowol, K. (2006) Chromatin modification and senescence: linkage by tumor suppressors? *Rejuvenation Res* 9, 69-76.
- Hankinson, O., Andersen, R.D., Birren, B.W., Sander, F., Negishi, M. and Nebert, D.W. (1985) Mutations affecting the regulation of transcription of the cytochrome P1-450 gene in the mouse Hepa-1 cell line. *J Biol Chem* 260, 1790-5.
- Hayes, J.D. and McLellan, L.I. (1999) Glutathione and glutathione-dependent enzymes represent a co-ordinately regulated defence against oxidative stress. *Free Radic Res* 31, 273-300.
- Heath-Pagliuso, S., Rogers, W.J., Tullis, K., Seidel, S.D., Cenijn, P.H., Brouwer, A. and Denison, M.S. (1998) Activation of the Ah receptor by tryptophan and tryptophan metabolites. *Biochemistry* 37, 11508-15.
- Heimler, I., Rawlins, R.G., Owen, H. and Hutz, R.J. (1998) Dioxin perturbs, in a dose- and time-dependent fashion, steroid secretion, and induces apoptosis of human luteinized granulosa cells. *Endocrinology* 139, 4373-9.
- Hohne, M., Becker-Rabbenstein, V., Kahl, G.F. and Taniguchi, H. (1990) Regulation of cytochrome P-450 CYP1A1 gene expression and proto-oncogene expression by growth factors in primary hepatocytes. *FEBS Lett* 273, 219-22.
- Hollenberg, P.F. (2002) Characteristics and common properties of inhibitors, inducers, and activators of CYP enzymes. *Drug Metab Rev* 34, 17-35.
- Hollingshead, B.D., Beischlag, T.V., Dinatale, B.C., Ramadoss, P. and Perdew, G.H. (2008) Inflammatory signaling and aryl hydrocarbon receptor mediate synergistic induction of interleukin 6 in MCF-7 cells. *Cancer Res* 68, 3609-17.
- Ikuta, T., Eguchi, H., Tachibana, T., Yoneda, Y. and Kawajiri, K. (1998) Nuclear localization and export signals of the human aryl hydrocarbon receptor. *J Biol Chem* 273, 2895-904.
- Ireland, R.C., Li, S.Y. and Dougherty, J.J. (1995) The DNA binding of purified Ah receptor heterodimer is regulated by redox conditions. *Arch Biochem Biophys* 319, 470-80.

- Itoh, K., Wakabayashi, N., Katoh, Y., Ishii, T., O'Connor, T. and Yamamoto, M. (2003) Keap1 regulates both cytoplasmic-nuclear shuttling and degradation of Nrf2 in response to electrophiles. *Genes Cells* 8, 379-91.
- Jain, S., Dolwick, K.M., Schmidt, J.V. and Bradfield, C.A. (1994) Potent transactivation domains of the Ah receptor and the Ah receptor nuclear translocator map to their carboxyl termini. *J Biol Chem* 269, 31518-24.
- Jiang, F., Zhang, Y. and Dusting, G.J. (2011) NADPH oxidase-mediated redox signaling: roles in cellular stress response, stress tolerance, and tissue repair. *Pharmacol Rev* 63, 218-42.
- Jomova, K. and Valko, M. (2011) Advances in metal-induced oxidative stress and human disease. *Toxicology* 283, 65-87.
- Jones, D.P. (2002) Redox potential of GSH/GSSG couple: assay and biological significance. *Methods Enzymol* 348, 93-112.
- Jonsson, M.E., Franks, D.G., Woodin, B.R., Jenny, M.J., Garrick, R.A., Behrendt, L., Hahn, M.E. and Stegeman, J.J. (2009) The tryptophan photoproduct 6-formylindolo[3,2-b]carbazole (FICZ) binds multiple AHRs and induces multiple CYP1 genes via AHR2 in zebrafish. *Chem Biol Interact* 181, 447-54.
- Kaminsky, L. (2006) The role of trace metals in cytochrome P4501 regulation. *Drug Metab Rev* 38, 227-34.
- Kazlauskas, A., Poellinger, L. and Pongratz, I. (1999) Evidence that the co-chaperone p23 regulates ligand responsiveness of the dioxin (Aryl hydrocarbon) receptor. *J Biol Chem* 274, 13519-24.
- Ke, S., Rabson, A.B., Germino, J.F., Gallo, M.A. and Tian, Y. (2001) Mechanism of suppression of cytochrome P-450 1A1 expression by tumor necrosis factor- α and lipopolysaccharide. *J Biol Chem* 276, 39638-44.
- Kensler, T.W., Wakabayashi, N. and Biswal, S. (2007) Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway. *Annu Rev Pharmacol Toxicol* 47, 89-116.
- Khor, T.O., Huang, M.T., Kwon, K.H., Chan, J.Y., Reddy, B.S. and Kong, A.N. (2006) Nrf2-deficient mice have an increased susceptibility to dextran sulfate sodium-induced colitis. *Cancer Res* 66, 11580-4.
- Kim, D.W., Gazourian, L., Quadri, S.A., Romieu-Mourez, R., Sherr, D.H. and Sonenshein, G.E. (2000) The RelA NF-kappaB subunit and the aryl hydrocarbon receptor (AhR) cooperate to transactivate the c-myc promoter in mammary cells. *Oncogene* 19, 5498-506.
- Kim, S.H., Henry, E.C., Kim, D.K., Kim, Y.H., Shin, K.J., Han, M.S., Lee, T.G., Kang, J.K., Gasiewicz, T.A., Ryu, S.H. and Suh, P.G. (2006) Novel compound 2-methyl-2H-pyrazole-3-carboxylic acid (2-methyl-4-o-tolylazo-phenyl)-amide (CH-223191) prevents 2,3,7,8-TCDD-induced toxicity by antagonizing the aryl hydrocarbon receptor. *Mol Pharmacol* 69, 1871-8.
- Kobayashi, A., Kang, M.I., Okawa, H., Ohtsuji, M., Zenke, Y., Chiba, T., Igarashi, K. and Yamamoto, M. (2004) Oxidative stress sensor Keap1 functions as an adaptor for Cul3-based E3 ligase to regulate proteasomal degradation of Nrf2. *Mol Cell Biol* 24, 7130-9.
- Kociba, R.J., Keyes, D.G., Beyer, J.E., Carreon, R.M., Wade, C.E., Dittenber, D.A., Kalnins, R.P., Frauson, L.E., Park, C.N., Barnard, S.D., Hummel, R.A. and Humiston, C.G. (1978) Results of a two-year chronic toxicity and oncogenicity study of 2,3,7,8-tetrachlorodibenzo-p-dioxin in rats. *Toxicol Appl Pharmacol* 46, 279-303.
- Lahvis, G.P. and Bradfield, C.A. (1998) Ahr null alleles: distinctive or different? *Biochem Pharmacol* 56, 781-7.
- Lahvis, G.P., Lindell, S.L., Thomas, R.S., McCuskey, R.S., Murphy, C., Glover, E., Bentz, M., Southard, J. and Bradfield, C.A. (2000) Portosystemic shunting and persistent fetal vascular structures in aryl hydrocarbon receptor-deficient mice. *Proc Natl Acad Sci U S A* 97, 10442-7.
- Lahvis, G.P., Pyzalski, R.W., Glover, E., Pitot, H.C., McElwee, M.K. and Bradfield, C.A. (2005) The aryl hydrocarbon receptor is required for developmental closure of the ductus venosus in the neonatal mouse. *Mol Pharmacol* 67, 714-20.

- Lambeth, J.D., Kawahara, T. and Diebold, B. (2007) Regulation of Nox and Duox enzymatic activity and expression. *Free Radic Biol Med* 43, 319-31.
- Lekas, P., Tin, K.L., Lee, C. and Prokipcak, R.D. (2000) The human cytochrome P450 1A1 mRNA is rapidly degraded in HepG2 cells. *Arch Biochem Biophys* 384, 311-8.
- Loignon, M., Miao, W., Hu, L., Bier, A., Bismar, T.A., Scrivens, P.J., Mann, K., Basik, M., Bouchard, A., Fiset, P.O., Batist, Z. and Batist, G. (2009) Cul3 overexpression depletes Nrf2 in breast cancer and is associated with sensitivity to carcinogens, to oxidative stress, and to chemotherapy. *Mol Cancer Ther* 8, 2432-40.
- Lorick, K.L., Toscano, D.L. and Toscano, W.A., Jr. (1998) 2,3,7,8-Tetrachlorodibenzo-p-dioxin alters retinoic acid receptor function in human keratinocytes. *Biochem Biophys Res Commun* 243, 749-52.
- Lu, S.C. (1999) Regulation of hepatic glutathione synthesis: current concepts and controversies. *FASEB J* 13, 1169-83.
- Lu, S.C. (2012) Glutathione synthesis. *Biochim Biophys Acta* 1830, 3143-53.
- Lu, Y.F., Santostefano, M., Cunningham, B.D., Threadgill, M.D. and Safe, S. (1995) Identification of 3'-methoxy-4'-nitroflavone as a pure aryl hydrocarbon (Ah) receptor antagonist and evidence for more than one form of the nuclear Ah receptor in MCF-7 human breast cancer cells. *Arch Biochem Biophys* 316, 470-7.
- Lushchak, V.I. (2011) Adaptive response to oxidative stress: Bacteria, fungi, plants and animals. *Comp Biochem Physiol C Toxicol Pharmacol* 153, 175-90.
- Ma, Q. (2011) Influence of light on aryl hydrocarbon receptor signaling and consequences in drug metabolism, physiology and disease. *Expert Opin Drug Metab Toxicol* 7, 1267-93.
- Ma, Q. and Baldwin, K.T. (2000) 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced degradation of aryl hydrocarbon receptor (AhR) by the ubiquitin-proteasome pathway. Role of the transcription activation and DNA binding of AhR. *J Biol Chem* 275, 8432-8.
- Ma, Q., Dong, L. and Whitlock, J.P., Jr. (1995) Transcriptional activation by the mouse Ah receptor. Interplay between multiple stimulatory and inhibitory functions. *J Biol Chem* 270, 12697-703.
- Ma, Q., Kinneer, K., Bi, Y., Chan, J.Y. and Kan, Y.W. (2004) Induction of murine NAD(P)H:quinone oxidoreductase by 2,3,7,8-tetrachlorodibenzo-p-dioxin requires the CNC (cap 'n' collar) basic leucine zipper transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2): cross-interaction between AhR (aryl hydrocarbon receptor) and Nrf2 signal transduction. *Biochem J* 377, 205-13.
- Ma, Q. and Whitlock, J.P., Jr. (1996) The aromatic hydrocarbon receptor modulates the Hepa 1c1c7 cell cycle and differentiated state independently of dioxin. *Mol Cell Biol* 16, 2144-50.
- Ma, Q. and Whitlock, J.P., Jr. (1997) A novel cytoplasmic protein that interacts with the Ah receptor, contains tetratricopeptide repeat motifs, and augments the transcriptional response to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *J Biol Chem* 272, 8878-84.
- Magiatis, P., Pappas, P., Gaitanis, G., Mexia, N., Melliou, E., Galanou, M., Vlachos, C., Stathopoulou, K., Skaltsounis, A.L., Marselos, M., Velegaki, A., Denison, M.S. and Bassukas, I.D. (2013) *Malassezia* Yeasts Produce a Collection of Exceptionally Potent Activators of the Ah (Dioxin) Receptor Detected in Diseased Human Skin. *J Invest Dermatol*. Paper in press.
- Mahadev, K., Motoshima, H., Wu, X., Ruddy, J.M., Arnold, R.S., Cheng, G., Lambeth, J.D. and Goldstein, B.J. (2004) The NAD(P)H oxidase homolog Nox4 modulates insulin-stimulated generation of H₂O₂ and plays an integral role in insulin signal transduction. *Mol Cell Biol* 24, 1844-54.
- Maher, J.M., Cherrington, N.J., Slitt, A.L. and Klaassen, C.D. (2006) Tissue distribution and induction of the rat multidrug resistance-associated proteins 5 and 6. *Life Sci* 78, 2219-25.

- Mahon, M.J. and Gasiewicz, T.A. (1995) Ah receptor phosphorylation: localization of phosphorylation sites to the C-terminal half of the protein. *Arch Biochem Biophys* 318, 166-74.
- Marlowe, J.L., Fan, Y., Chang, X., Peng, L., Knudsen, E.S., Xia, Y. and Puga, A. (2008) The aryl hydrocarbon receptor binds to E2F1 and inhibits E2F1-induced apoptosis. *Mol Biol Cell* 19, 3263-71.
- Martyn, K.D., Frederick, L.M., von Loehneysen, K., Dinanuer, M.C. and Knaus, U.G. (2006) Functional analysis of Nox4 reveals unique characteristics compared to other NADPH oxidases. *Cell Signal* 18, 69-82.
- Matsumura, F. (2003) On the significance of the role of cellular stress response reactions in the toxic actions of dioxin. *Biochem Pharmacol* 66, 527-40.
- Matsumura, F. and Vogel, C.F. (2006) Evidence supporting the hypothesis that one of the main functions of the aryl hydrocarbon receptor is mediation of cell stress responses. *Biol Chem* 387, 1189-94.
- McGuire, J., Whitelaw, M.L., Pongratz, I., Gustafsson, J.A. and Poellinger, L. (1994) A cellular factor stimulates ligand-dependent release of hsp90 from the basic helix-loop-helix dioxin receptor. *Mol Cell Biol* 14, 2438-46.
- McIntosh, B.E., Hogenesch, J.B. and Bradfield, C.A. (2010) Mammalian Per-Arnt-Sim proteins in environmental adaptation. *Annu Rev Physiol* 72, 625-45.
- Meister, A. and Anderson, M.E. (1983) Glutathione. *Annu Rev Biochem* 52, 711-60.
- Meyer, B.K. and Perdew, G.H. (1999) Characterization of the AhR-hsp90-XAP2 core complex and the role of the immunophilin-related protein XAP2 in AhR stabilization. *Biochemistry* 38, 8907-17.
- Meyer, B.K., Petrusis, J.R. and Perdew, G.H. (2000) Aryl hydrocarbon (Ah) receptor levels are selectively modulated by hsp90-associated immunophilin homolog XAP2. *Cell Stress Chaperones* 5, 243-54.
- Mikstacka, R., Przybylska, D., Rimando, A.M. and Baer-Dubowska, W. (2007) Inhibition of human recombinant cytochromes P450 CYP1A1 and CYP1B1 by trans-resveratrol methyl ethers. *Mol Nutr Food Res* 51, 517-24.
- Mimura, J., Ema, M., Sogawa, K. and Fujii-Kuriyama, Y. (1999) Identification of a novel mechanism of regulation of Ah (dioxin) receptor function. *Genes Dev* 13, 20-5.
- Mimura, J., Yamashita, K., Nakamura, K., Morita, M., Takagi, T.N., Nakao, K., Ema, M., Sogawa, K., Yasuda, M., Katsuki, M. and Fujii-Kuriyama, Y. (1997) Loss of teratogenic response to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in mice lacking the Ah (dioxin) receptor. *Genes Cells* 2, 645-54.
- Moorthy, B., Parker, K.M., Smith, C.V., Bend, J.R. and Welty, S.E. (2000) Potentiation of oxygen-induced lung injury in rats by the mechanism-based cytochrome P-450 inhibitor, 1-aminobenzotriazole. *J Pharmacol Exp Ther* 292, 553-60.
- Morel, Y. and Barouki, R. (1998) Down-regulation of cytochrome P450 1A1 gene promoter by oxidative stress. Critical contribution of nuclear factor 1. *J Biol Chem* 273, 26969-76.
- Moriarty-Craige, S.E. and Jones, D.P. (2004) Extracellular thiols and thiol/disulfide redox in metabolism. *Annu Rev Nutr* 24, 481-509.
- Mukai, M. and Tischkau, S.A. (2007) Effects of tryptophan photoproducts in the circadian timing system: searching for a physiological role for aryl hydrocarbon receptor. *Toxicol Sci* 95, 172-81.
- Muller, G.F., Dohr, O., El-Bahay, C., Kahl, R. and Abel, J. (2000) Effect of transforming growth factor-beta1 on cytochrome P450 expression: inhibition of CYP1 mRNA and protein expression in primary rat hepatocytes. *Arch Toxicol* 74, 145-52.
- Muntane-Relat, J., Ourlin, J.C., Domergue, J. and Maurel, P. (1995) Differential effects of cytokines on the inducible expression of CYP1A1, CYP1A2, and CYP3A4 in human hepatocytes in primary culture. *Hepatology* 22, 1143-53.
- Nebert, D.W. and Dalton, T.P. (2006) The role of cytochrome P450 enzymes in endogenous signalling pathways and environmental carcinogenesis. *Nat Rev Cancer* 6, 947-60.
- Negishi, M., Pedersen, L.G., Petrotchenko, E., Shevtsov, S., Gorokhov, A., Kakuta, Y. and Pedersen, L.C. (2001) Structure and function of sulfotransferases. *Arch Biochem Biophys* 390, 149-57.

- Nguyen, L.P. and Bradfield, C.A. (2008) The search for endogenous activators of the aryl hydrocarbon receptor. *Chem Res Toxicol* 21, 102-16.
- Nguyen, T., Nioi, P. and Pickett, C.B. (2009) The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress. *J Biol Chem* 284, 13291-5.
- Nguyen, T., Sherratt, P.J., Huang, H.C., Yang, C.S. and Pickett, C.B. (2003) Increased protein stability as a mechanism that enhances Nrf2-mediated transcriptional activation of the antioxidant response element. Degradation of Nrf2 by the 26 S proteasome. *J Biol Chem* 278, 4536-41.
- Oberg, M., Bergander, L., Hakansson, H., Rannug, U. and Rannug, A. (2005) Identification of the tryptophan photoproduct 6-formylindolo[3,2-b]carbazole, in cell culture medium, as a factor that controls the background aryl hydrocarbon receptor activity. *Toxicol Sci* 85, 935-43.
- Oesch-Bartlomowicz, B. and Oesch, F. (2005) Phosphorylation of cytochromes P450: first discovery of a posttranslational modification of a drug-metabolizing enzyme. *Biochem Biophys Res Commun* 338, 446-9.
- Oetari, S., Sudibyo, M., Commandeur, J.N., Samhoedi, R. and Vermeulen, N.P. (1996) Effects of curcumin on cytochrome P450 and glutathione S-transferase activities in rat liver. *Biochem Pharmacol* 51, 39-45.
- Ohtake, F., Takeyama, K., Matsumoto, T., Kitagawa, H., Yamamoto, Y., Nohara, K., Tohyama, C., Krust, A., Mimura, J., Chambon, P., Yanagisawa, J., Fujii-Kuriyama, Y. and Kato, S. (2003) Modulation of oestrogen receptor signalling by association with the activated dioxin receptor. *Nature* 423, 545-50.
- Oliveira, M., Santos, M.A. and Pacheco, M. (2004) Glutathione protects heavy metal-induced inhibition of hepatic microsomal ethoxyresorufin O-deethylase activity in *Dicentrarchus labrax* L. *Ecotoxicol Environ Saf* 58, 379-85.
- Omiecinski, C.J., Vanden Heuvel, J.P., Perdew, G.H. and Peters, J.M. (2010) Xenobiotic metabolism, disposition, and regulation by receptors: from biochemical phenomenon to predictors of major toxicities. *Toxicol Sci* 120 Suppl 1, S49-75.
- Paine, A.J. (1976) Induction of benzo[a]pyrene Mono-oxygenase in liver cell culture by the photochemical generation of active oxygen species. Evidence for the involvement of singlet oxygen and the formation of a stable inducing intermediate. *Biochem J* 158, 109-17.
- Park, H.S., Jung, H.Y., Park, E.Y., Kim, J., Lee, W.J. and Bae, Y.S. (2004) Cutting edge: direct interaction of TLR4 with NAD(P)H oxidase 4 isozyme is essential for lipopolysaccharide-induced production of reactive oxygen species and activation of NF-kappa B. *J Immunol* 173, 3589-93.
- Parkinson, A. (1996) Casarett and Doull 's Toxicology: The basic science of poisons. ed. C.D. Klassen, Kansas City.
- Pendyala, S., Gorshkova, I.A., Usatyuk, P.V., He, D., Pennathur, A., Lambeth, J.D., Thannickal, V.J. and Natarajan, V. (2009) Role of Nox4 and Nox2 in hyperoxia-induced reactive oxygen species generation and migration of human lung endothelial cells. *Antioxid Redox Signal* 11, 747-64.
- Perdew, G.H. (1988) Association of the Ah receptor with the 90-kDa heat shock protein. *J Biol Chem* 263, 13802-5.
- Peterson, K.J. and Butterfield, N.J. (2005) Origin of the Eumetazoa: testing ecological predictions of molecular clocks against the Proterozoic fossil record. *Proc Natl Acad Sci U S A* 102, 9547-52.
- Petry, A., Djordjevic, T., Weitnauer, M., Kietzmann, T., Hess, J. and Gorlach, A. (2006) NOX2 and NOX4 mediate proliferative response in endothelial cells. *Antioxid Redox Signal* 8, 1473-84.
- Pohl, C., Will, F., Dietrich, H. and Schrenk, D. (2006) Cytochrome P450 1A1 expression and activity in Caco-2 cells: modulation by apple juice extract and certain apple polyphenols. *J Agric Food Chem* 54, 10262-8.
- Poland, A., Glover, E. and Kende, A.S. (1976) 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* 251, 4936-46.

- Poland, A. and Knutson, J.C. (1982) 2,3,7,8-tetrachlorodibenzo-p-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. *Annu Rev Pharmacol Toxicol* 22, 517-54.
- Pollenz, R.S. (2002) The mechanism of AH receptor protein down-regulation (degradation) and its impact on AH receptor-mediated gene regulation. *Chem Biol Interact* 141, 41-61.
- Pollenz, R.S., Davarinis, N.A. and Shearer, T.P. (1999) Analysis of aryl hydrocarbon receptor-mediated signaling during physiological hypoxia reveals lack of competition for the aryl hydrocarbon nuclear translocator transcription factor. *Mol Pharmacol* 56, 1127-37.
- Pongratz, I., Mason, G.G. and Poellinger, L. (1992) Dual roles of the 90-kDa heat shock protein hsp90 in modulating functional activities of the dioxin receptor. Evidence that the dioxin receptor functionally belongs to a subclass of nuclear receptors which require hsp90 both for ligand binding activity and repression of intrinsic DNA binding activity. *J Biol Chem* 267, 13728-34.
- Probst, M.R., Reisz-Porszasz, S., Agbunag, R.V., Ong, M.S. and Hankinson, O. (1993) Role of the aryl hydrocarbon receptor nuclear translocator protein in aryl hydrocarbon (dioxin) receptor action. *Mol Pharmacol* 44, 511-8.
- Ramos-Gomez, M., Kwak, M.K., Dolan, P.M., Itoh, K., Yamamoto, M., Talalay, P. and Kensler, T.W. (2001) Sensitivity to carcinogenesis is increased and chemoprotective efficacy of enzyme inducers is lost in nrf2 transcription factor-deficient mice. *Proc Natl Acad Sci U S A* 98, 3410-5.
- Rangasamy, T., Cho, C.Y., Thimmulappa, R.K., Zhen, L., Srisuma, S.S., Kensler, T.W., Yamamoto, M., Petrache, I., Tudor, R.M. and Biswal, S. (2004) Genetic ablation of Nrf2 enhances susceptibility to cigarette smoke-induced emphysema in mice. *J Clin Invest* 114, 1248-59.
- Rannug, A., Rannug, U., Rosenkranz, H.S., Winqvist, L., Westerholm, R., Agurell, E. and Grafstrom, A.K. (1987) Certain photooxidized derivatives of tryptophan bind with very high affinity to the Ah receptor and are likely to be endogenous signal substances. *J Biol Chem* 262, 15422-7.
- Reyes, H., Reisz-Porszasz, S. and Hankinson, O. (1992) Identification of the Ah receptor nuclear translocator protein (Arnt) as a component of the DNA binding form of the Ah receptor. *Science* 256, 1193-5.
- Rhee, S.G. (2006) Cell signaling. H₂O₂, a necessary evil for cell signaling. *Science* 312, 1882-3.
- Roberts, B.J. and Whitelaw, M.L. (1999) Degradation of the basic helix-loop-helix/Per-ARNT-Sim homology domain dioxin receptor via the ubiquitin/proteasome pathway. *J Biol Chem* 274, 36351-6.
- Rockwell, P., Martinez, J., Papa, L. and Gomes, E. (2004) Redox regulates COX-2 upregulation and cell death in the neuronal response to cadmium. *Cell Signal* 16, 343-53.
- Sadek, C.M. and Allen-Hoffmann, B.L. (1994) Cytochrome P450IA1 is rapidly induced in normal human keratinocytes in the absence of xenobiotics. *J Biol Chem* 269, 16067-74.
- Schalldach, C.M., Riby, J. and Bjeldanes, L.F. (1999) Lipoxin A4: a new class of ligand for the Ah receptor. *Biochemistry* 38, 7594-600.
- Schmidt, J.V., Su, G.H., Reddy, J.K., Simon, M.C. and Bradfield, C.A. (1996) Characterization of a murine Ahr null allele: involvement of the Ah receptor in hepatic growth and development. *Proc Natl Acad Sci U S A* 93, 6731-6.
- Schroder, K., Zhang, M., Benkhoff, S., Mieth, A., Pliquett, R., Kosowski, J., Kruse, C., Luedike, P., Michaelis, U.R., Weissmann, N., Dimmeler, S., Shah, A.M. and Brandes, R.P. (2012) Nox4 is a protective reactive oxygen species generating vascular NADPH oxidase. *Circ Res* 110, 1217-25.
- Seelig, G.F., Simonsen, R.P. and Meister, A. (1984) Reversible dissociation of gamma-glutamylcysteine synthetase into two subunits. *J Biol Chem* 259, 9345-7.
- Seidel, S.D., Winters, G.M., Rogers, W.J., Ziccardi, M.H., Li, V., Keser, B. and Denison, M.S. (2001) Activation of the Ah receptor signaling pathway by prostaglandins. *J Biochem Mol Toxicol* 15, 187-96.

- Sergent, T., Dupont, I., Jassogne, C., Ribonnet, L., van der Heiden, E., Scippo, M.L., Muller, M., McAlister, D., Pussemier, L., Larondelle, Y. and Schneider, Y.J. (2009a) CYP1A1 induction and CYP3A4 inhibition by the fungicide imazalil in the human intestinal Caco-2 cells-comparison with other conazole pesticides. *Toxicol Lett* 184, 159-68.
- Sergent, T., Dupont, I., Van der Heiden, E., Scippo, M.L., Pussemier, L., Larondelle, Y. and Schneider, Y.J. (2009b) CYP1A1 and CYP3A4 modulation by dietary flavonoids in human intestinal Caco-2 cells. *Toxicol Lett* 191, 216-22.
- Shahidi, F. and Wanasundara, P.K. (1992) Phenolic antioxidants. *Crit Rev Food Sci Nutr* 32, 67-103.
- Shetty, P.V., Bhagwat, B.Y. and Chan, W.K. (2003) P23 enhances the formation of the aryl hydrocarbon receptor-DNA complex. *Biochem Pharmacol* 65, 941-8.
- Shimada, T. (2006) Xenobiotic-metabolizing enzymes involved in activation and detoxification of carcinogenic polycyclic aromatic hydrocarbons. *Drug Metab Pharmacokinet* 21, 257-76.
- Shimada, T., Tanaka, K., Takenaka, S., Murayama, N., Martin, M.V., Foroozesh, M.K., Yamazaki, H., Guengerich, F.P. and Komori, M. (2010) Structure-function relationships of inhibition of human cytochromes P450 1A1, 1A2, 1B1, 2C9, and 3A4 by 33 flavonoid derivatives. *Chem Res Toxicol* 23, 1921-35.
- Shin, S., Wakabayashi, N., Misra, V., Biswal, S., Lee, G.H., Agoston, E.S., Yamamoto, M. and Kensler, T.W. (2007) NRF2 modulates aryl hydrocarbon receptor signaling: influence on adipogenesis. *Mol Cell Biol* 27, 7188-97.
- Sim, E., Lack, N., Wang, C.J., Long, H., Westwood, I., Fullam, E. and Kawamura, A. (2008) Arylamine N-acetyltransferases: structural and functional implications of polymorphisms. *Toxicology* 254, 170-83.
- Singh, S.S., Hord, N.G. and Perdew, G.H. (1996) Characterization of the activated form of the aryl hydrocarbon receptor in the nucleus of HeLa cells in the absence of exogenous ligand. *Arch Biochem Biophys* 329, 47-55.
- Smith, K.R., Klei, L.R. and Barchowsky, A. (2001) Arsenite stimulates plasma membrane NADPH oxidase in vascular endothelial cells. *Am J Physiol Lung Cell Mol Physiol* 280, L442-9.
- Song, Z. and Pollenz, R.S. (2002) Ligand-dependent and independent modulation of aryl hydrocarbon receptor localization, degradation, and gene regulation. *Mol Pharmacol* 62, 806-16.
- Song, Z. and Pollenz, R.S. (2003) Functional analysis of murine aryl hydrocarbon (AH) receptors defective in nuclear import: impact on AH receptor degradation and gene regulation. *Mol Pharmacol* 63, 597-606.
- Spink, D.C., Lincoln, D.W., 2nd, Dickerman, H.W. and Gierthy, J.F. (1990) 2,3,7,8-Tetrachlorodibenzo-p-dioxin causes an extensive alteration of 17 beta-estradiol metabolism in MCF-7 breast tumor cells. *Proc Natl Acad Sci U S A* 87, 6917-21.
- Sturrock, A., Cahill, B., Norman, K., Huecksteadt, T.P., Hill, K., Sanders, K., Karwande, S.V., Stringham, J.C., Bull, D.A., Gleich, M., Kennedy, T.P. and Hoidal, J.R. (2006) Transforming growth factor-beta1 induces Nox4 NAD(P)H oxidase and reactive oxygen species-dependent proliferation in human pulmonary artery smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 290, L661-L673.
- Sumimoto, H., Hata, K., Mizuki, K., Ito, T., Kage, Y., Sakaki, Y., Fukumaki, Y., Nakamura, M. and Takeshige, K. (1996) Assembly and activation of the phagocyte NADPH oxidase. Specific interaction of the N-terminal Src homology 3 domain of p47phox with p22phox is required for activation of the NADPH oxidase. *J Biol Chem* 271, 22152-8.
- Suzuki, T. and Nohara, K. (2007) Regulatory factors involved in species-specific modulation of arylhydrocarbon receptor (AhR)-dependent gene expression in humans and mice. *J Biochem* 142, 443-52.
- Swanson, H.I. (2002) DNA binding and protein interactions of the AHR/ARNT heterodimer that facilitate gene activation. *Chem Biol Interact* 141, 63-76.
- Talalay, P., Dinkova-Kostova, A.T. and Holtzclaw, W.D. (2003) Importance of phase 2 gene regulation in protection against electrophile and reactive oxygen toxicity and carcinogenesis. *Adv Enzyme Regul* 43, 121-34.

- Tang, T., Lin, X., Yang, H., Zhou, L., Wang, Z., Shan, G. and Guo, Z. (2010) Overexpression of antioxidant enzymes upregulates aryl hydrocarbon receptor expression via increased Sp1 DNA-binding activity. *Free Radic Biol Med* 49, 487-92.
- Taylor, B.L. and Zhulin, I.B. (1999) PAS domains: internal sensors of oxygen, redox potential, and light. *Microbiol Mol Biol Rev* 63, 479-506.
- Taylor, R.T., Wang, F., Hsu, E.L. and Hankinson, O. (2009) Roles of coactivator proteins in dioxin induction of CYP1A1 and CYP1B1 in human breast cancer cells. *Toxicol Sci* 107, 1-8.
- Thum, T. and Borlak, J. (2004) Mechanistic role of cytochrome P450 monooxygenases in oxidized low-density lipoprotein-induced vascular injury: therapy through LOX-1 receptor antagonism? *Circ Res* 94, e1-13.
- Tian, Y., Ke, S., Denison, M.S., Rabson, A.B. and Gallo, M.A. (1999) Ah receptor and NF-kappaB interactions, a potential mechanism for dioxin toxicity. *J Biol Chem* 274, 510-5.
- Tian, Y., Ke, S., Thomas, T., Meeker, R.J. and Gallo, M.A. (1998) Transcriptional suppression of estrogen receptor gene expression by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). *J Steroid Biochem Mol Biol* 67, 17-24.
- Ueng, Y.F., Jan, W.C., Lin, L.C., Chen, T.L., Guengerich, F.P. and Chen, C.F. (2002) The alkaloid rutaecarpine is a selective inhibitor of cytochrome P450 1A in mouse and human liver microsomes. *Drug Metab Dispos* 30, 349-53.
- Uno, S., Dalton, T.P., Derkenne, S., Curran, C.P., Miller, M.L., Shertzer, H.G. and Nebert, D.W. (2004) Oral exposure to benzo[a]pyrene in the mouse: detoxication by inducible cytochrome P450 is more important than metabolic activation. *Mol Pharmacol* 65, 1225-37.
- Uno, S., Dalton, T.P., Dragin, N., Curran, C.P., Derkenne, S., Miller, M.L., Shertzer, H.G., Gonzalez, F.J. and Nebert, D.W. (2006) Oral benzo[a]pyrene in Cyp1 knockout mouse lines: CYP1A1 important in detoxication, CYP1B1 metabolism required for immune damage independent of total-body burden and clearance rate. *Mol Pharmacol* 69, 1103-14.
- Van den Berg, M., Birnbaum, L.S., 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. and Peterson, R.E. (2006) The 2005 World Health Organization reevaluation of human and Mammalian toxic equivalency factors for dioxins and dioxin-like compounds. *Toxicol Sci* 93, 223-41.
- Vasquez, A., Atallah-Yunes, N., Smith, F.C., You, X., Chase, S.E., Silverstone, A.E. and Vikstrom, K.L. (2003) A role for the aryl hydrocarbon receptor in cardiac physiology and function as demonstrated by AhR knockout mice. *Cardiovasc Toxicol* 3, 153-63.
- Vernhet, L., Allain, N., Le Vee, M., Morel, F., Guillouzo, A. and Fardel, O. (2003) Blockage of multidrug resistance-associated proteins potentiates the inhibitory effects of arsenic trioxide on CYP1A1 induction by polycyclic aromatic hydrocarbons. *J Pharmacol Exp Ther* 304, 145-55.
- Wakabayashi, N., Dinkova-Kostova, A.T., Holtzclaw, W.D., Kang, M.I., Kobayashi, A., Yamamoto, M., Kensler, T.W. and Talalay, P. (2004) Protection against electrophile and oxidant stress by induction of the phase 2 response: fate of cysteines of the Keap1 sensor modified by inducers. *Proc Natl Acad Sci U S A* 101, 2040-5.
- Waller, C.L. and McKinney, J.D. (1995) Three-dimensional quantitative structure-activity relationships of dioxins and dioxin-like compounds: model validation and Ah receptor characterization. *Chem Res Toxicol* 8, 847-58.
- Wang, Z., Yang, H., Ramesh, A., Roberts, L.J., 2nd, Zhou, L., Lin, X., Zhao, Y. and Guo, Z. (2009) Overexpression of Cu/Zn-superoxide dismutase and/or catalase accelerates benzo(a)pyrene detoxification by upregulation of the aryl hydrocarbon receptor in mouse endothelial cells. *Free Radic Biol Med* 47, 1221-9.
- Wanner, R., Brommer, S., Czarnetzki, B.M. and Rosenbach, T. (1995) The differentiation-related upregulation of aryl hydrocarbon receptor transcript

- levels is suppressed by retinoic acid. *Biochem Biophys Res Commun* 209, 706-11.
- Wei, Y.D., Helleberg, H., Rannug, U. and Rannug, A. (1998) Rapid and transient induction of CYP1A1 gene expression in human cells by the tryptophan photoproduct 6-formylindolo[3,2-b]carbazole. *Chem Biol Interact* 110, 39-55.
- Weiss, C., Kolluri, S.K., Kiefer, F. and Gottlicher, M. (1996) Complementation of Ah receptor deficiency in hepatoma cells: negative feedback regulation and cell cycle control by the Ah receptor. *Exp Cell Res* 226, 154-63.
- Wells, P.G., Mackenzie, P.I., Chowdhury, J.R., Guillemette, C., Gregory, P.A., Ishii, Y., Hansen, A.J., Kessler, F.K., Kim, P.M., Chowdhury, N.R. and Ritter, J.K. (2004) Glucuronidation and the UDP-glucuronosyltransferases in health and disease. *Drug Metab Dispos* 32, 281-90.
- White, S.S. and Birnbaum, L.S. (2009) An overview of the effects of dioxins and dioxin-like compounds on vertebrates, as documented in human and ecological epidemiology. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev* 27, 197-211.
- Whitelaw, M., Pongratz, I., Wilhelmsson, A., Gustafsson, J.A. and Poellinger, L. (1993) Ligand-dependent recruitment of the Arnt coregulator determines DNA recognition by the dioxin receptor. *Mol Cell Biol* 13, 2504-14.
- Whitelaw, M.L., Gustafsson, J.A. and Poellinger, L. (1994) Identification of transactivation and repression functions of the dioxin receptor and its basic helix-loop-helix/PAS partner factor Arnt: inducible versus constitutive modes of regulation. *Mol Cell Biol* 14, 8343-55.
- Whitlock, J.P., Jr. (1999) Induction of cytochrome P4501A1. *Annu Rev Pharmacol Toxicol* 39, 103-25.
- Wilhelmsson, A., Cuthill, S., Denis, M., Wikstrom, A.C., Gustafsson, J.A. and Poellinger, L. (1990) The specific DNA binding activity of the dioxin receptor is modulated by the 90 kd heat shock protein. *EMBO J* 9, 69-76.
- Wincent, E., Amini, N., Luecke, S., Glatt, H., Bergman, J., Crescenzi, C., Rannug, A. and Rannug, U. (2009) The suggested physiologic aryl hydrocarbon receptor activator and cytochrome P4501 substrate 6-formylindolo[3,2-b]carbazole is present in humans. *J Biol Chem* 284, 2690-6.
- Xu, C., Siu, C.S. and Pasco, D.S. (1998) DNA binding activity of the aryl hydrocarbon receptor is sensitive to redox changes in intact cells. *Arch Biochem Biophys* 358, 149-56.
- Yeager, R.L., Reisman, S.A., Aleksunes, L.M. and Klaassen, C.D. (2009) Introducing the "TCDD-inducible AhR-Nrf2 gene battery". *Toxicol Sci* 111, 238-46.
- Young, J.C., Moarefi, I. and Hartl, F.U. (2001) Hsp90: a specialized but essential protein-folding tool. *J Cell Biol* 154, 267-73.
- Zhang, S., Qin, C. and Safe, S.H. (2003) Flavonoids as aryl hydrocarbon receptor agonists/antagonists: effects of structure and cell context. *Environ Health Perspect* 111, 1877-82.
- Zhang, S., Rowlands, C. and Safe, S. (2008) Ligand-dependent interactions of the Ah receptor with coactivators in a mammalian two-hybrid assay. *Toxicol Appl Pharmacol* 227, 196-206.
- Zhao, B., Degroot, D.E., Hayashi, A., He, G. and Denison, M.S. (2010) CH223191 is a ligand-selective antagonist of the Ah (Dioxin) receptor. *Toxicol Sci* 117, 393-403.
- Zhen, L., King, A.A., Xiao, Y., Chanock, S.J., Orkin, S.H. and Dinanuer, M.C. (1993) Gene targeting of X chromosome-linked chronic granulomatous disease locus in a human myeloid leukemia cell line and rescue by expression of recombinant gp91phox. *Proc Natl Acad Sci U S A* 90, 9832-6.