

Alternative Negative Feedback Control in the Aryl Hydrocarbon Receptor Signaling Pathway

Jason Matthews^{1,2*}

¹Department of Pharmacology and Toxicology, University of Toronto, Toronto, Ontario, M5S1A8, Canada

²Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo, 1046 Blindern, 0316 Oslo, Norway

The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor and member of the basic helix-loop-helix Period-ARNT (AHR nuclear translocator)-Single-minded protein family. The AHR is best known for mediating the toxic effects of the environmental contaminant, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Some of which include dysregulated lipid and glucose metabolism, carcinogenesis and dysfunction of the immune, reproductive and nervous systems [1]. However, the precise molecular mechanisms are not well established. AHR also regulates responses of numerous anthropogenic and natural chemicals, including several endogenous and dietary compounds [2], most of which do not cause TCDD-like toxicity. Recently, additional roles for AHR in adaptive drug metabolism, differentiation, vasculature development and in the modulation of regulatory T cells (Tregs) and proinflammatory T helper 17 (Th17) cells have been reported [3,4]. These findings have prompted intense interest in exploring AHR as a therapeutic target for cancer, autoimmune and other human diseases [5]. Understanding the molecular mechanisms of AHR signaling will provide a better appreciation of its biological role, its utility as a therapeutic target and the relationship between TCDD-exposure and human disease.

In the presence of bound ligand, cytoplasmic AHR translocates to the nucleus and heterodimerizes with ARNT. The activated AHR/ARNT heterodimer binds to aryl hydrocarbon response elements (AHREs; also known as xenobiotic response elements [XREs]) in the regulatory regions of its target genes, commonly known as the “AHR gene battery” [6]. The genomic era continues to identify a seemingly endless list of AHR target genes, including xenobiotic drug metabolizing enzymes cytochrome P450 1A1 (CYP1A1) and CYP1B1, as well as genes involved in cell cycle, differentiation and development [7]. Most, but not all, genomic regions bound by AHR contain AHREs, suggesting that AHR is more flexible in its DNA binding preference or that it is recruited to DNA through protein tethering to other DNA bound transcription factors [8].

Although several studies have reported a wide-range of cellular activities regulated by AHR, there has been much less focus on determining how AHR-mediated responses are controlled. Currently, AHR signaling can be down-regulated by arguably three different mechanisms. There are a number of natural and synthetic AHR antagonists, including resveratrol, found in red wine and dietary flavonoids, such as kaempferol, which inhibit AHR recruitment to CYP1A1 and consequently TCDD-induced AHR transactivation [9]. Another well-known mechanism to down-regulate AHR signaling is through ligand-induced proteolytic degradation [10]. After entering the nucleus, agonist-activated AHR is exported and degraded by the ubiquitin/proteasome pathway. This degradation, however, is influenced by cell context and is not observed with some AHR agonists [10]. Similar to other PAS proteins, the AHR signaling pathway contains an auto-regulatory feedback loop, mediated by another member of the PAS family referred to as the aryl hydrocarbon receptor repressor (AHRR) [11]. AHRR is structurally similar to AHR, but it does not bind ligand and it lacks a transcriptional activation domain. Overexpression

studies strongly support AHRR as a ligand-induced repressor of AHR [11,12]. It was originally proposed that AHRR bound and sequestered ARNT resulting in the quenching of AHR transactivation [11]. However, AHRR was recently reported to interact directly with AHR and overexpression of ARNT failed to rescue AHRR-dependent repression of AHR [12]. Repression of CYP1 activity in human skin fibroblast was not correlated with AHRR expression levels [13]. A similar lack of correlation was reported between AHRR expression and CYP1A1 responsiveness in mice exposed to the AHR agonist, benzo[α]pyrene [14]. It is important to note that these studies reported AHRR mRNA but not protein levels. Ahrr-deficient mice exhibited tissue-specific increases in CYP1A1 mRNA induction compared with wild-type mice [15], suggesting that alternative mechanisms regulate AHR transactivation in other tissues. The effect of AHRR loss on the expression of other genes in the “AHR gene battery” has not been reported.

In an effort to find alternative AHR target genes that might influence AHR transactivation, we began studying TCDD-inducible poly (ADP-ribose) polymerase (TiPARP aka ARTD14) [16]. TiPARP is a member of the poly(ADP-ribose) polymerase family that use nicotinamide adenine dinucleotide (NAD⁺) as a substrate to catalyze the transfer of individual ADP-ribose units (referred to as mono-ADP-ribosyltransferase activity) and/or polymerization ADP-ribose onto target proteins [17]. ADP-ribosylation is important for many cellular activities including DNA repair, transcription, apoptosis, proliferation and cell death [18]. We recently found that TiPARP exhibited mono-ADP-ribosyltransferase rather than poly(ADP-ribose) polymerase activity [19]. More interesting were the findings that RNAi-mediated knockdown of TiPARP increased, whereas TiPARP overexpression repressed TCDD-induced CYP1A1 and CYP1B1 mRNA levels. TCDD-dependent induction of AHR target genes, including AHRR was also enhanced in immortalized Tiparp^{-/-} mouse embryonic fibroblasts (MEFs) compared with wild-type cells. We also observed higher AHR protein levels and reduced TCDD-dependent proteolytic degradation in human cells exposed to RNAi targeting TiPARP and Tiparp^{-/-} MEFs compared with control and wild-type cells, respectively [19]. Interesting, the ability of TiPARP to repress AHR signaling also

***Corresponding author:** Jason Matthews, Department of Pharmacology and Toxicology, University of Toronto, Toronto, Ontario, M5S1A8, Canada, Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo, 1046 Blindern, 0316 Oslo, Norway, Tel: 416-946-0851; Fax: 416-978-6395; E-mail: Jason.matthews@utoronto.ca

Received December 14, 2012; **Accepted** December 17, 2012; **Published** December 19, 2012

Citation: Matthews J (2013) Alternative Negative Feedback Control in the Aryl Hydrocarbon Receptor Signaling Pathway. J Drug Metab Toxicol 4: e116. doi:10.4172/2157-7609.1000e116

Copyright: © 2013 Matthews J. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

required an intact catalytic domain [19]. However, the molecular targets of TipARP and whether this enzyme post translationally modifies AHR or other components of the AHR signaling pathway remains to be determined. Post-translational modifications of transcription factors play important roles in regulating their activity, stability and ability to interact or recruit co-activator or co-repressor proteins. Phosphorylation of AHR is important for its ability to interact with AHREs and regulate reporter gene expression [20]. As mentioned above, AHR is ubiquitinated and proteolytically degraded. Similar to AHRR and ARNT, AHR has recently been reported to be sumoylated [21]. Sumoylation of AHR was reversed after TCDD treatment, suggesting that sumoylation may play an important role in regulated constitutive or ligand independent function. It will be important to identify the cellular targets of TipARP and determine if AHR is subject to ADP-ribosylation as well as how ADP-ribosylation may influence the ubiquitination and/or sumoylation of AHR.

Collectively, the recent analysis of TipARP reveal that it is a negative regulator of AHR signaling and part of a new negative feedback loop involving the proteolytic degradation of AHR. It will be important to determine the specificity of TipARP to repress AHR transactivation and if TipARP functions as a transcription repressor for other transcription factors. Careful analysis of the influence of TipARP loss on the “AHR gene battery” will be essential to determine the impact of TipARP as a global repressor of AHR transactivation. Moreover, comprehensive studies of Tiparp^{-/-} mice will be necessary to confirm the *in vitro* repressive function of TipARP as well as determine whether Tiparp^{-/-} mice also exhibit increased sensitivity to TCDD toxicity. TipARP may represent an alternative therapeutic target to modulate AHR signaling in human diseases.

References

- Birnbaum LS (1994) The mechanism of dioxin toxicity: relationship to risk assessment. Environ Health Perspect 102: 157-167.
- Denison MS, Nagy SR (2003) Activation of the aryl hydrocarbon receptor by structurally diverse exogenous and endogenous chemicals. Annu Rev Pharmacol Toxicol 43: 309-334.
- Veldhoen M, Hirota K, Westendorf AM, Buer J, Dumoutier L, et al. (2008) The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. Nature 453: 106-109.
- Quintana FJ, Basso AS, Iglesias AH, Korn T, Farez MF, et al. (2008) Control of T(reg) and T(H)17 cell differentiation by the aryl hydrocarbon receptor. Nature 453: 65-71.
- Pot C (2012) Aryl hydrocarbon receptor controls regulatory CD4⁺ T cell function. Swiss Med Wkly 142: 13592.
- Nebert DW, Puga A, Vasiliou V (1993) Role of the Ah receptor and the dioxin-inducible [Ah] gene battery in toxicity, cancer, and signal transduction. Ann N Y Acad Sci 685: 624-640.
- Lo R, Celius T, Forgacs AL, Dere E, MacPherson L, et al. (2011) Identification of aryl hydrocarbon receptor binding targets in mouse hepatic tissue treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol Appl Pharmacol 257: 38-47.
- Lo R, Matthews J (2012) High-Resolution Genome-wide Mapping of AHR and ARNT Binding Sites by ChIP-Seq. Toxicol Sci 130: 349-361.
- Macpherson L, Matthews J (2010) Inhibition of aryl hydrocarbon receptor-dependent transcription by resveratrol or kaempferol is independent of estrogen receptor alpha expression in human breast cancer cells. Cancer Lett 299: 119-129.
- Pollenz RS, Buggy C (2006) Ligand-dependent and -independent degradation of the human aryl hydrocarbon receptor (hAHR) in cell culture models. Chem Biol Interact 164: 49-59.
- Mimura J, Ema M, Sogawa K, Fujii-Kuriyama Y (1999) Identification of a novel mechanism of regulation of Ah (dioxin) receptor function. Genes Dev 13: 20-25.
- Karchner SI, Jenny MJ, Tarrant AM, Evans BR, Kang HJ, et al. (2009) The active form of human aryl hydrocarbon receptor (AHR) repressor lacks exon 8, and its Pro 185 and Ala 185 variants repress both AHR and hypoxia-inducible factor. Mol Cell Biol 29: 3465-3477.
- Tigges J, Weighardt H, Wolff S, Götz C, Förster I, et al. (2012) Aryl Hydrocarbon Receptor Repressor (AhRR) Function Revisited: Repression of CYP1 Activity in Human Skin Fibroblasts Is Not Related to AhRR Expression. J Invest Dermatol 133: 87-96.
- Bernshausen T, Jux B, Esser C, Abel J, Fritsche E (2006) Tissue distribution and function of the Aryl hydrocarbon receptor repressor (AhRR) in C57BL/6 and Aryl hydrocarbon receptor deficient mice. Arch Toxicol 80: 206-211.
- Hosoya T, Harada N, Mimura J, Motohashi H, Takahashi S, et al. (2008) Inducibility of cytochrome P450 1A1 and chemical carcinogenesis by benzo[a]pyrene in AhR repressor-deficient mice. Biochem Biophys Res Commun 365: 562-567.
- Ma Q, Baldwin KT, Renzelli AJ, McDaniel A, Dong L (2001) TCDD-inducible poly(ADP-ribose) polymerase: a novel response to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Biochem Biophys Res Commun 289: 499-506.
- Hassa PO, Haenni SS, Elser M, Hottiger MO (2006) Nuclear ADP-ribosylation reactions in mammalian cells: where are we today and where are we going? Microbiol Mol Biol Rev 70: 789-829.
- Schreiber V, Dantzer F, Ame JC, de Murcia G (2006) Poly (ADP-ribose): novel functions for an old molecule. Nat Rev Mol Cell Biol 7: 517-528.
- MacPherson L, Tamblyn L, Rajendra S, Bralha F, McPherson JP, et al. (in press) 2,3,7,8-tetrachlorodibenzo-p-dioxin poly(ADP-ribose) polymerase (TipARP, ARTD14) is a mono-ADP-ribosyltransferase and repressor of aryl hydrocarbon receptor transactivation. Nucleic Acids Res.
- Pongratz I, Strömstedt PE, Mason GG, Poellinger L (1991) Inhibition of the specific DNA binding activity of the dioxin receptor by phosphatase treatment. J Biol Chem 266: 16813-16817.
- Xing X, Bi H, Chang AK, Zang MX, Wang M, et al. (2012) SUMOylation of AhR modulates its activity and stability through inhibiting its ubiquitination. J Cell Physiol 227: 3812-3819.