MECHANISM OF SIGNAL TRANSDUCTION BY THE DIOXIN RECEPTOR

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Abstract

The dioxin / aryl hydrocarbon receptor functions as a ligand-activated transcription factor regulating transcription of a battery of genes encoding drug metabolizing enzymes. A series of independent loss-of-function (gene disruption) studies in mice have not yielded unequivocal results with regard to a potential physiological function of the dioxin receptor. We have generated a gain-of-function mouse model to assess potential biological functions of the receptor. To more precisely understand the mode of regulation of the dioxin receptor we have also performed mechanistic experiments to elucidate the mechanism of repression of receptor function in the absence of ligand (where planar environmental pollutants constitute high-affinity ligands) and the mechanism of de-repression in the presence of ligand.

Introduction

The dioxin receptor (aryl hydrocarbon) receptor [AhR] is a ligand-inducible transcription factor that mediates cellular response to xenobiotic compounds such as environmental pollutants, e.g. polycyclic aromatic hydrocarbons and polychlorinated dioxins, most notably 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). The receptor belongs to the basic helix-loop-helix (bHLH)/Per-ARNT-Sim domain (PAS) family of transcription factors (for review, see[1]). This family also contains gene regulatory proteins involved in regulation of circadian rhythmicity and hypoxia-inducible transcription factors. Thus, This family of proteins is emerging as a battery of regulatory factors seemingly designed to respond to environmental cues. Interestingly, a recent report has indicated that the same ligands that activate transcriptional responses by the dioxin receptor can also induce a function of the receptor as an E3 ubiquitin ligase upon recruitment of necessary cofactors[2].

A series of independent loss-of-function (gene disruption) studies in mice have not yielded unequivocal results with regard to a potential physiological function of the dioxin receptor. In addition, there is no conclusive information concerning physiological ligands of the dioxin receptor. Environmental pollutants such as planar dioxins, biphenyls and polyaromatic hydrocarbons represent the best characterized receptor ligands. Consistent with the uncertainty regarding the physiological function of the receptor there is a paucity of information on target genes of the receptor that are not drug metabolizing enzymes. Although a series of different gene expression profiling studies have recently indicated dysregulation of numerous genes upon exposure of cells to dioxin, the relevance of these genes for dioxin toxicity and carcinogenicity in vivo remains unclear.

Results and Discussion

Two conserved domains characterize the dioxin receptor and bHLH/PAS proteins in general - the N-terminal bHLH DNA binding domain and the PAS domain, which spans two hydrophobic repeats termed PAS-A and PAS-B. The dioxin receptor is unique among bHLH/PAS proteins, because it contains a ligand-binding region located in the C-terminal part of the PAS domain including the PAS-B motif. In the absence of ligand, the latent receptor is associated with the molecular chaperone Hsp90 and two Hsp90-binding proteins, the co-chaperone p23, and XAP2[3-6]. Hsp90 maintains a high affinity ligand-binding conformation of the receptor, whereas p23 is thought to stabilize the latent receptor-Hsp90 heterocomplex. XAP2, in turn, stabilizes the AhR protein[6] and participates in regulation of the intracellular localization of the receptor by an uncharacterized cytoplasmic retention mechanism[6,7]. Upon ligand binding, AhR accumulates in the nucleus where it forms a transcriptionally active complex with the bHLH/PAS transcription factor ARNT. Dimerization with ARNT induces the release of the Hsp90 complex from the receptor. The AhR-ARNT heterodimer activates the transcription of target genes by specific binding to xenobiotic-inducible transcriptional control elements, XREs. They are located in the

regulatory regions of a gene network encoding drug-metabolizing enzymes such as cytochrome P-4501A1[1].

Activation of the dioxin receptor-mediated signaling processes by xenobiotic compounds in animal model systems (mainly in rodents) has numerous toxic consequences, including carcinogenesis, immunosuppression, thymic involution, severe wasting and death[1]. As outlined above, a series of independent loss-of-function (gene disruption) studies in mice have not yielded unequivocal results with regard to a potential physiological function of the dioxin receptor. In contrast, we have developed a gain-of –function model to assess potential biological functions of the dioxin receptor system. To this end, we have generated transgenic mice expressing a constitutively active dioxin receptor. These mice (CA-AhR mice) develop invasive tumors of the glandular stomach from 3-4 months of age that correlate with increased mortality beginning at 6-9 months of age[8]. This mouse model differs, however, from experimentally-induced models of gastric tumors in that the CA-AhR mice were not exposed to any carcinogen or viral oncogene. The tumors lack the cellular attributes of malignancy and no metastasis can be observed although increased mortality is observed in CA-AhR mice, beginning at 6 months of age. Both the severity of the tumors at a certain age and increased mortality were influenced both by the sex of the animals as well as the CA-AhR founder line[8]. Taken together, these data indicate that the dioxin receptor may have an important biological function in regulation of gastro-intestinal epithelial cell homeostasis. Possibly, this process may be modulated by planar ligands that are derived from the diet, most notably planar indole derivatives such as indolo-3-carbinol[1].

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