MECHANISMS OF TOXICOLOGICAL AND PHYSIOLOGICAL ACTIONS OF ARYLHYDROCARBON RECEPTOR (AHR) IN MICE

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Abstract

Arylhydrocarbon receptor (AhR or dioxin receptor, DR) was originally identified as a cytoplasmic factor which binds TCDD and was supposed to mediate induction of drug-metabolizing enzymes (DME) by TCDD. cDNA cloning and subsequent biochemical analysis revealed that AhR belongs to a super-gene family of transcription factor structurally characterized by basic helix- loop- helix (bHLH) and PAS domains, and function as transcription factor of *CYP1A1* gene in response to xenobiotics such as TCDD.

Functional analyses of AhR-deficient mice revealed that most of toxicological effects, if not all, caused by TCDD such as teratogenesis and tumor promotion, were mediated by AhR. However, recent close investigations into the loss of functions in the AhR deficient mice have begun to reveal that AhR is involved in multiple facets of normal animal physiology, such as reproduction, hepatic angiogenesis, immunology and intestinal cell proliferation.

Introduction

AhR was originally indentified as a cytoplasmic factor, which bound TCDD with a high affinity and was supposed to mediate the induction of xenobiotic-metabolizing CYP1A1.

Molecular biological studies revealed the mechanisms of AhR functions in the induction of CYP1A1 in response to xeboliotics including TCDD and 3-methylcholanthrene (3MC): AhR is a member of a super-gene family of transcription factors with characteristic structural motifs of bHLH (basic helix-loop-helix) and PAS (per, Arnt and Sim homologous) domains. Under normal conditions, AhR occurs in the cytoplasm in a complex with HSP90, XAP and p23. When TCDD is taken up in the cells and bound with AhR in the complex, the liganded AhR translocates to the nuclei where it forms a heterodimer with Arnt, another bHLH-PAS protein and binds the XRE (xenobiotic responsive element) sequence in the promoter of the target genes resulting in their enhanced expression [1].

Functional analysis of AhR-KO mice revealed that toxicological effects caused by TCDD, such as teratogenesis, tumor promotion, immunosuppression due to thymic involution and hepatotoxicity were mediated by AhR. Despite the fact that AhR mediates these multiple adverse effects caused by TCDD, AhR is well conserved across a variety of animal species from nematodes to mammals, suggesting that AhR plays an important role in animal physiology. Indeed, recent detailed investigations into the loss of functions of the AhR-KO mice have begun to reveal that AhR is involved in the multiple facets of the normal animal physiology. Here we will discuss our recent findings of the roles of AhR in female reproduction and in suppression of colon tumorigenesis.

Material and Methods

AhR-KO mice were generated by homologous recombination as described previously and were kept under SPF or germ-free conditions. Gel mobility shift assay was carried out according to the standard method. Human embryonic kidney-derived 293 cells and other cultured cells were grown in DMEM (Sigma, St. Louis, Mo.) supplemented with 10% fetal bovine serum at 37˚C in 5% CO_2. Cells were plated at approximately 15% confluence 1 day before transfection. Immunohistochemistry using appropriate antibodies was performed as described previously and Chip assay was conducted according to the standard method with slight modifications.

All animal experiments were approved by the Institutional Animal Use Committee of the University.

Results and Discussion

AhR structure and mechanisms of transcriptional activation by AhR

By using the XRE-binding property and a partial N-terminal aminoacid sequence, we isolated cDNA clone of AhR from a cDNA library of Hepa-1 cells. Sequence analysis of the cDNA revealed that the encoded aminoacid sequence of AhR consists of 805 aminoacid residues and contains characteristic structural motifs of bHLH and PAS domains which are shared by Arnt (Ahr nuclear translocator) whose cDNA clone was previously isolated by Hankinson et al. as a cofactor of AhR [1].

Cell biological studies including our and other groups demonstrated that under normal conditions, AhR exists in the cytoplasm in a complex containing HSP90, XAP2 and p23. Upon binding with a ligand such as TCDD, AhR in the complex translocates to nuclei where it forms a heterodimer with Arnt and binds the XRE sequence in the promoter of its target genes to enhance their expression [1].

Toxiclogical role of AhR

In order to investigate the functions of AhR, three groups generated independently AhR-KO mice by homologous recombination and found that AhR mediates various toxicological effects caused by TCDD, such as cleft palate, hydronephrosis, tumor promotion, immunosuppression due to thymic involution and hepatotoxicity, because AhR-KO mice became resistant to these effects [2].

Physiological role of AhR

Over the past decade since the experiments using AhR-KO mice were first reported, many studies have examined AhR as a mediator of the adverse and toxicological response to environmental pollutants, such as 3MC

and TCDD. However, high degree of evolutional conservation of AhR across a variety of animal species from nematode to mammals suggest that AhR may possess important xenobiotic-independent and physiological functions.

Close investigation into the loss of function of AhR-KO mice revealed that the reproductive activities of the male and female mice were significantly reduced: especially female reproductive activity of AhR-KO mice was quntitated to be reduced by 2 to 3 folds in term of pup size. Folliculogenesis apparently normally developed up to a preovulatory stage, but ovulation stage was found defective in the AhR-KO mice. The number of ovulated oocytes was reduced by approximately 6 folds in the ovary of AhR-KO mice and formation of corpus luteum was rarely observed in the AhR-KO mice. Since these defective phenotypes were similarly observed in the CYP19-null or ER-null mice, we were interested to determine the concentrations of estradiol (E_2) in the ovaries of the wildtype and AhR-KO mice. The ovarian E_2 concentrations in the AhR-KO mince were lowered to one third to one fifth of those in the wildtype mice. Normally, CYP19 expression was changed cyclically with a peak at a preovulatory stage during the estrous cycle of 4 to 5 days, while it remained low at a basal level without a peak at a preovulatory stage in the AhR-KO female mice. Survey of the regulatory sequences in the upstream of the *CYP19* gene indentified the XRE and the Ad4 sequences in the mouse, human and fish. We constructed a reporter gene by fusing the upstream promoter sequence of *CYP19* gene and luciferase structural gene and transfected it into a cultured cell line CV-1 together with effector plasmids of AhR or Ad4BP, or both. While an effector plasmid of either alone enhanced slightly expression of the reporter gene, their combined transfection enhanced synergistically the reporter gene expression. It was also clearly demonstrated by the ChIP (Chromatin immunoprecipitation) analysis using granulosa cells that AhR and Ad4BP interacted with each other on the chromatin to enhance the expression of *CYP19* gene. Supplement with E_2 significantly recovered the pup size of the AhR-KO female mice, in support of the fact that reduced synthesis of E_2 is at least one of the causes of the defective reproduction of the AhR-KO mice [3].

Tumor suppressor function of AhR in colon

AhR-KO mice frequently suffered from prolapses at 20 weeks of age or later with about 50% of the total. Pathological analysis of gut revealed that neoplasmic lesions occurred in the colon of the AhR-KO mice. By thorough examination of digestive tracts, we found that AhR-KO mice developed colonic tumors especially at the cecum, whereas neither AhR heterozygous nor wildtype mice developed these tumors. Tumors started to be found in 30% of AhR-KO mice at 8 weeks of age and the incidence of the tumor bearing increased to 100% of the AhR-KO mice at 11 weeks. The tumor size increased gradually by age and reached a plateau at 30 weeks of age. The colon cancers that developed were predominantly classified into tubular adenocarcinomas of well-, moderately- and poorly-differentiated types, sometimes showing the invasion into submucosal regions or beyond. Overall survival rate estimated by using the Kaplan-Meyer method revealed that AhR-KO mice showed a significantly shorter longevity than that of the wildtype or heterozygous mice.

To examine the molecular mechanism of the development of colon cancers in the AhR-KO mice, we analyzed the expression of both AhR and β-catenin in the intestines of the wildtype and AhR-KO mice at an age of 6 weeks whose intestines were composed of apparently normal epithelia. AhR showed a relatively abundant

expression in Paneth cells of the AhR wildtype mice. Notably, β-catenin was abnormally accumulated in epithelial cells of the small intestine and especially in the nuclei of paneth cells of the AhR-KO mice compared with the counterparts in wildtype mice. Overexpression of β-catenin was also observed with the epithelial cells of cecum and colon of the AhR-KO mice. Using immunoblot analysis, we confirmed a high level of β-catenin in the cecum of AhR-KO mice compared with that of the AhR wildtype mice, whereas β-catenin mRNA expressions were not altered between cecums of the wildtype and the AhR-KO mice, suggestive of the stabilization of β-catenin protein in the AhR-KO mouse intestine. Recently we have reported that the AhR/Arnt heterodimer functions as a scaffold of ubiquitination complex containing Cul4B and DDB-1 for ER and AR [4]. We examined whether AhR/Arnt plays also a role in ubiquitination of β-catenin leading to its degradation by proteasome. *In vitro* and *in vivo* experiments using a reconstituted system of ubiquitination and MG132 used as inhibitor, the AhR and Arnt heterodimer was involved in the ubiquitination and subsequent proteasome degradation of β-catenin in a ligand-dependent manner which is different from that of the APC system. These results demonstrate that AhR functions as tumor suppressor by degradation of β-catenin independent of the APC system.

Reference

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