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Regulation of aryl hydrocarbon receptor-mediated transcription in human retinal pigmented epithelial cells

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ABSTRACT

The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor with pleiotropic effects in normal physiology or vascular development, xenobiotic metabolism, and cancer. A previous study has reported that BRG1, a component of the SWI/SNF complex, is a coactivator for AHR and is recruited to the promoter region of the *CYP1A1* gene in mouse hepatocytes. Recent data suggest that AHR is also expressed in human retinal pigment epithelial cells (ARPE-19), which play a crucial role in retinal physiology and the visual cycle. Multiple studies have shown that the AHR plays an important role in the pathogenesis of retinal diseases including age-related macular degeneration. However, the mechanism of AHR transcriptional activation in retinal pigment cells has not been reported. Here, we demonstrate that the AHR signaling pathway is active in ARPE-19 cells, as in hepatocytes, but with different target gene specificity. We also found that chromatin remodeling by the BRG1-containing SWI/SNF complex is required for the AHR-mediated expression of target genes in ARPE-19 cells. We identified a novel enhancer region (−12 kb) of the *CYP1A1* gene in ARPE-19 cells, to which both AHR and BRG1 are recruited in a ligand-dependent manner. BRG1 is associated with the AHR in ARPE-19 cells, and the C-terminal activation domain of the AHR directly interacts with BRG1. Furthermore, depletion of BRG1 caused a reduction in chromatin accessibility at the *CYP1A1* enhancer. These results suggest that ARPE-19 cells possess an AHR-mediated transcription pathway with different target gene specificity, and that BRG1 is required for AHR-mediated transcription in ARPE-19 cells.

1. Introduction

The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that belongs to the basic helix-loop-helix/Per-Arnt-Sim (bHLH/PAS) family of proteins [1]. The AHR is important in mediating cellular responses to a variety of environmental contaminants such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and benzo[a]pyrene [2]. Ligand binding enables the translocation of the AHR into the nucleus, and after heterodimerization with the AHR nuclear translocator (ARNT), the AHR/ARNT complex initiates transcription of a variety of AHR target genes with promoters containing xenobiotic responsive elements [3]. AHR target genes include cytochrome P450 (*CYP*) enzymes such as *CYP1A1*, *CYP1A2*, and *CYP1B1* that are important in the bioactivation of carcinogens and metabolism, and some phase-2 detoxification enzymes such as aldehyde dehydrogenase 3A1 (*ALDH3A1*) in liver [4]. Functionally, the AHR has been shown to be involved in normal physiology or vascular development, angiogenesis, and cancer [5,6]. Although $Ahr^{-/-}$ mice are viable and fertile, they exhibit a number of defects in liver development and increased proliferative and inflammatory marker gene expression compared to $Ahr^{+/+}$ mice [2,7].

Recent studies have shown that the AHR is expressed at relatively high levels in a human retinal pigment epithelial (RPE) cell line (ARPE-19), primary cell lines derived from adult donors, and RPE cells isolated from freshly obtained adult donor tissue [8,9]. The retinal pigment epithelium is a monolayer of pigmented cells that play a crucial role in retinal physiology by forming a part of the blood-retina barrier and supporting photoreceptor function [10]. In regard to AHR signaling in RPE cells, dysfunction of its signaling pathway in adult mice leads to abnormal eye movements in the form of a spontaneous pendular horizontal nystagmus [11], and subretinal accumulation of microglia and focal RPE atrophy [12]. AHR knockdown was sufficient to increase collagen type IV production and secretion in RPE cell cultures along with increased expression of angiogenic and inflammatory molecules, including

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chemokine (C-C motif) ligand 2 and vascular endothelial growth factor A [13].

Recently, a relationship between AHR signaling in RPE cells and dry-type age-related macular degeneration (AMD) has been proposed [9]. It has been demonstrated that AHR activity and protein levels in RPE cells are gradually decreased with advanced age, eventually leading to decreases in cellular xenobiotic metabolism and matrix metabolism, resulting in accumulation of extracellular matrix molecules and debris (sub-RPE deposits), as seen in dry-type AMD [9].

Indeed, aged *Ahr*$^{-/-}$ mice exhibit decreased visual function and hallmark characteristics of dry-type AMD. Furthermore, decreased AHR activity promotes sub-RPE deposit formation, through the effects of increased oxidized low-density lipoprotein on RPE cells [9]. Moreover, decreased expression and activity of AHR increases choroidal endothelial cell migration and tube formation *in vivo* and exacerbates murine neovascular AMD [13].

Although AHR-mediated gene expression has been shown to be involved in the development of AMD, the exact mechanism underlying the transcriptional activation by AHR in RPE cells is largely absent and remains to be determined. In the present study, we demonstrate that the AHR signaling pathway is active in human RPE cells, but with different target gene specificity from that in hepatocytes. Chromatin immunoprecipitation analysis demonstrated that AHR and BRG1 are recruited to the enhancer region of the *CYP1A1* gene in a TCDD-dependent manner in ARPE-19 cells. In addition, BRG1 is required for TCDD-mediated transcription of the *CYP1A1* gene and chromatin accessibility at the *CYP1A1* enhancer region. These results provide a potential mechanism for the expression of the AHR target genes in ARPE-19 cells.

2. Materials and methods

2.1. Plasmids

The hemagglutinin (HA)-tagged mouse AHR (pACTAG2) and AHR(N) (amino acids 1–374) and AHR(C) (amino acids 375–805) fragments have been reported previously [14] and were provided by Dr. Jeong Hoon Kim (Sungkyunkwan University, Seoul, Korea).

2.2. Cell culture

Human RPE cells (ARPE-19) and hepatoma cells (HepG2) were purchased from American Type Culture Collection (Manassas, VA, USA). Cells were grown as a monolayer in Dulbecco's modified Eagle's medium F-12 (ARPE-19) obtained from Welgene (Daegu, Korea) or in Dulbecco's modified Eagle's medium (HepG2), supplemented with 10% fetal bovine serum (FBS) at 37 °C and in an atmosphere containing 5% CO_2.

2.3. RNA interference

Small-interfering RNA (siRNA) experiments were performed according to a previously published method [15]. The sequences of siRNAs used were siBRG1, 5′-CCGUGGACUUCAAGAAGAUdTdT-3′ (sense) and 5′-AUCUUCUUGAAGUCCACGGdTdT-3′ (antisense); siBRM, 5′-CCAGTAGGCAGGAAACCGAAGdTdT-3′ (sense) and 5′-GGCTTGCATATGGCGATACAdTdT-3′ (antisense); nonspecific siRNA (siNS), 5′-UUCUCCGAACGUGUCACGUdTdT-3′ (sense) and 5′-ACGUGACACGUUCGGAGAAdTdT-3′ (antisense). siRNAs were transfected into ARPE-19 and HepG2 cells by using Oligofectamine (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's protocol.

2.4. Quantitative RT-PCR (RT-qPCR)

Total RNA was isolated from ARPE-19 and HepG2 cells with Trizol (Invitrogen, Carlsbad, CA, USA) after TCDD treatments as indicated, and reverse transcribed using the iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA, USA). Real-time PCR amplification of this cDNA was performed using a Roche Light-Cycler 480 II and SYBR Green Master Mix (Roche, Indianapolis, IN, USA). The primers used were as follows: *CYP1A1*, 5′-TGAACCC-CAGGGTACAGAGA-3′ (forward) and 5′-GGCCTCCATATAGGGCAGAT-3′ (reverse); *CYP1A2*, 5′-AATCAGGTGGTGGTGTCAGT-3′ (forward) and 5′- ACTCTCAGGGAAGTGCTGTC-3′ (reverse); *AHRR* (AHR repressor), 5′-AGACTCCAGGACCCACAAAG-3′ (forward) and 5′-CATCCTCACTGTGCTTTCCC-3′ (reverse). Relative expression levels were normalized to *18s* mRNA levels. Results shown are means and the range of variation of duplicate PCR reactions performed on the same cDNA sample; the results are from a single experiment that is representative of at least two independent experiments conducted on different days.

2.5. Protein binding assay

For the co-immunoprecipitation assay, extracts from ARPE-19 cells treated with TCDD or vehicle were prepared in 1.0 mL of radioimmunoprecipitation assay (RIPA) buffer (50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 2 mM EDTA, 1% SDS, 1% sodium deoxycholate, and 1% NP-40). Immunoblotting was performed as described previously by using the following antibodies: anti-BRG1, anti-AHR, normal mouse IgG (all from Santa Cruz Biotechnology, Dallas, TX, USA). For *in vitro* binding assays using HA-fused AHR, N-terminal and C-terminal proteins were respectively expressed *in vitro* using TNT Quick Coupled Transcription/Translation Systems (Promega, Madison, WI, USA). ARPE-19 cells were treated in the absence or presence of TCDD for 1 h, incubated with TNT lysates, and immunoprecipitated by antibody/protein A Sepharose.

2.6. Chromatin immunoprecipitation (ChIP) assay

ChIP assays were performed according to previously described protocols [15]. Immunoprecipitation was conducted with anti-AHR (Abcam, Cambridge, UK), anti-BRG1 (Epigentek, Farmingdale, NY, USA). Sequences of ChIP primers are listed in Supplementary Table 1. Results are expressed as percent of input chromatin (before immunoprecipitation).

2.7. Formaldehyde-assisted isolation of regulatory elements (FAIRE)-qPCR

FAIRE-qPCR was performed as previously described [16]. Cells were treated with 10 nM TCDD or toluene for 24 h. Results shown are means and the range of variation of duplicate PCR reactions performed on the same DNA sample. Results are expressed as percentage of input chromatin (Input) and were derived from a single experiment that is representative of at least two independent experiments. Sequences of FAIRE-qPCR primers are the same as those used for ChIP-qPCR analysis in Supplementary Table 1.

3. Results

3.1. AHR-mediated transcription in ARPE-19 cells

Previous studies have shown that primary RPE cell lines derived from adult donors and the human RPE cell line ARPE-19 express high levels of AHR [8,9]. To determine whether the AHR-mediated transcription is functional in human RPE cells, we assessed the