Supplemental Materials and Methods

Real time PCR primers for mRNA expression

Gene	5´-forward primer-3´	5´-reverse primer-3´
IL6 mRNA	aaattcggtacatcctcgacggca	agtgcctctttgctgctttcacac
GAPDH mRNA	tgcaccaccaactgcttagc	ggcatggactgtggtcatgag
CYP1A1 mRNA	tcttccttcgtccccttcac	tggttgatctgccactggtt

PCR primers for ChIP analysis of IL6 gene

IL6 promoter region	5´-forward primer-3´	5´-reverse primer-3´
transcription start site	cggtgggctcgagggcagaatgagc	gttctgcttcttagcgctagcc
-500 bp	tgcgatggagtcagaggaaactca	agacatgccaaagtgctgagtcac
-700 bp	agcactggcagcacaaggcaaac	caagcctgggattatgaagaagg
-1.0 kb	agtggtgaagagactcagtggcaa	tttgaggatggccaggcagttcta
-1.5 kb	caacaggcgggtcctgaaatgtta	gcccatttgcatgagaccaaggat
-2.0 kb	cctgcatgccctgatgtcctattt	cagtggcttcgtttcatgcaggaa
-2.5 kb	gagcaagacgcaagctggactaat	tgaaccgatatagccgagctggaa
-3.0 kb	acaaagaatgagctctccacgcga	acacacatgccttcccacagtttg
-3.5 kb	actcccatttgtctggcctctctt	tcacgcctgtaaacccagcacttt
-4.0 kb	ctccaacatcagctggctctttct	attctggcagcaactgcaagttcc
-4.5 kb	tgcaatcagggcactctcttccaa	tgagaaacacagcagggcagatgt
-5.0 kb	aaggaggcttagttcagagggact	gaaaccagccaatgctttgcatgc

Antibodies for western blotting

AHR: Rpt1 (Thermo Scientific), p23 (JJ3 Asciites was provided by David Toft, The Mayo Clinic Rochester MN), ARNT (Affinity Bioreagents; MA1-515), RELB (Santa Cruz; sc-226), HDAC1 (Santa Cruz; sc-7872), HDAC3 (Santa Cruz; sc-11417)

Antibodies for ChIP

ARNT (Affinity Bioreagents; MA1-515), RNA polymerase II (Upstate; CTD4H8), p65 (Santa Cruz; sc-109), p50 (Santa Cruz; sc-7178X), K310 acetylated p65 (Cell Signaling; 3045S), IKBζ (Santa Cruz; sc-66935), IKKα (Santa Cruz; sc-7182), IKKβ (Santa Cruz; sc-34673), HDAC1 (Santa Cruz; sc-7872), HDAC3 (Santa Cruz; sc-11417), NCOR (Santa Cruz; sc-8994), SIN3A (Affinity Bioreagents; PA1-870), SMRT (Santa Cruz; sc-1610), K4 dimethylated histone H3 (Millipore; 07-030), K4 trimethylated histone H3 (Millipore; 04-745), S10 phosphorylated histone H3 (Millipore; 04-817), K4 mono, di, trimethylated histone H3 (Upstate; 05-791), acetylated histone H3 (Upstate; 06-599), C/EBPβ (Santa Cruz; sc-150), CJUN (Santa Cruz; sc-1694), CBP (Santa Cruz; sc-369), BRG1 (Santa Cruz; sc-10768)

Production of AHR antibody

A polyclonal rabbit serum was produced against a peptide corresponding to the human AHR sequence GTEHLRKRNTKLPFMFTTGEAVLYEATNC (amino acid residues 391-418) was

produced by Thermo Scientific (Affinity BioReagents). The carboxyl cysteine residue was added to the carboxyl terminus of the peptide in order to couple peptide to bovine serum albumin. This sequence has previously been shown to be at or near the XAP2 binding site on the AHR (1). Antibodies to this peptide were isolated by affinity chromatography using peptide coupled to maleimide-activated BSA (Thermo Scientific). This conjugate was then coupled to Affi-gel 10 gel (Bio-Rad).

Preparation of AHR-GS construct.

A synthetic human *AHR* cDNA sequence optimized for mammalian codon use and minimal secondary mRNA structure was purchased from GenScript (Piscataway, NJ). This cDNA was inserted into the pcDNA3 expression plasmid. Using the QuikChange Site-Directed Mutagenesis Kit (Stratagene. Palo Alto, CA) according to manufacturer's instructions, nucleotides encoding the amino acids glycine and serine (i.e., GGA TCC) were inserted into the cDNA. When coded for protein this addition will result in glycine and serine being inserted between arginine 40 and aspartic acid 41 of the wild-type AHR protein sequence, which are part of the DNA-binding-domain of the protein. The primers used for this procedure were *(forward)* 5´-AGCAAACGGCACCGGGGATCCGATCGGCTGAACACC-3´ and *(reverse)* 5´-GGTGTTCAGCCGATCGGATCCCCGGTGCCGTTTGCT-3´. The addition of nucleotides was confirmed by sequencing. A similar mutation was described previously for the mouse AHR (2).

Characterization of AHR-GS construct

Cell culture and luciferase reporter assay: COS-1 cells were maintained in DMEM with 10% fetal bovine serum, 2 mM glutamine, 10 mM HEPES, 0.15% sodium bicarbonate, 50 units/ml penicillin, and 50 μg/ml streptomycin, and transfected in the same media containing 8% dextran/charcoal treated FBS in place of the normal FBS. Cells were incubated at 37°C with 5% CO_2. For the assay, COS-1 cells were seeded in 6-well plates. Once the cells reached ~80% confluency they were transiently transfected with pDJM/βgal (200 ng/well), pGudLuc6.1 (200 ng/well), and plasmids (20 ng/well) containing either hAHR or synthetic hAHR-GS using Lipofectamine (Invitrogen, Carlsbad, CA) according to manufacturer's protocols. The following day cells were treated with DMSO or TCDD (10 nM) for 4 h. Cells were lysed and luciferase activity was determined using Luciferase Assay System Substrate (Promega, Madison, WI) according to manufacturer's directions. Transfection efficiency was determined by measuring βgal activity for all of the samples.

Immunoblotting: COS-1 cells were grown in 100-mm dishes and transfected with 4.5 μg of plasmid. Whole-cell extracts were prepared by lysing cells in MENG buffer [25 mM MOPS, 2 mM EDTA, 0.02% NaN_3, 10% glycerol, 1% NP40, pH 7.4] supplemented with 20 mM Na_2MoO_4 and protease inhibitor cocktail (Sigma). Homogenates were centrifuged at 42,000 x g for 1 h at 4^oC, and the soluble fraction was collected. Protein concentrations were determined using the BCA protein assay kit (Thermo Scientific, Rockford, IL). Protein samples were resolved by Tricine SDS-PAGE and transferred to PVDF membrane. Immunoblotting was performed using antibodies directed against AHR (3) and XAP2 (mAb anti-ARA9, NB 100-127, Novus Biologicals, Littleton, CO). Proteins were visualized using biotin-conjugated secondary antibodies (Jackson Immunoresearch, West Grove, PA) in conjunction with ^{125}I streptavidin.

Ligand binding assay: Assays were performed as described previously (4) using cell homogenates prepared from COS-1 cells transfected with AHR or AHR-GS, as described above.

Electrophoretic Mobility Shift Assay: Assays were performed as described previously (54) using plasmids containing hAHR or AHR-GS with the TNT Coupled Reticulocyte Lysate System to prepare *in vitro* translated proteins. Indirubin was used at a concentration of 1 μM.

References

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4. Flaveny, C. A. , Murray, I. A. , Chiaro, C. R. , and Perdew, G. H. (2009) *Mol Pharmacol*. **75**(6), 1412-20