

Establishment of a stable aryl hydrocarbon receptor-responsive HepG2 cell line

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Received: 1 October 2013 / Accepted: 20 February 2014 / Published online: 26 March 2014
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Abstract The aryl hydrocarbon receptor (AHR) is a ligand-dependent transcription factor. It heterodimerizes with aryl hydrocarbon nuclear translocator, binds to the xenobiotic-responsive element (XRE), and enhances the transcription of genes encoding xenobiotic metabolizing enzymes. AHR also plays important roles in the inhibition of intestinal carcinogenesis and the modulation of gut immunity. It is very important to screen for AHR-activating compounds because those are expected to produce the AHR-mediated physiological functions. Until now, AHR-mediated transcriptional activity represented by the transcriptional activity of CYP1A1 in luciferase assay has been applied as a screening procedure for AHR-activating compounds. However, the AHR-mediated transcriptional activity did not necessarily correspond with the CYP1A1 transcriptional activity. To evaluate AHR-

mediated transcriptional activity more specifically, and to screen for AHR-activating compounds, we establish a stable AHR-responsive HepG2 cell line by co-transfection of an AHR expression vector and an AHR-responsive vector (pGL3-XRE) containing a luciferase gene and three tandemly arranged XRE elements into a human hepatoma derived cell line, HepG2. The induction of luciferase activity in the stable AHR-responsive HepG2 cell line by typical AHR activators occurred in time- and concentration-dependent manners. By assessing the AHR target genes CYP1A1, UGT1A1, and ABCG2, an AHR activator-mediated induction was observed at mRNA level. Furthermore, the AHR activator-mediated induction of luciferase activity was positively correlated with the mRNA levels of CYP1A1, UGT1A1, and ABCG2. These findings verified the usefulness of the established stable AHR-responsive HepG2 cell line for the screening of AHR-activating compounds.

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Keywords Aryl hydrocarbon receptor ·
Luciferase assay · CYP1A1 · UGT1A1 · ABCG2

Introduction

The aryl hydrocarbon receptor (AHR) is a ligand-dependent transcription factor that is widely represented in human cells and tissues (Pohjanvirta and Tuomisto 1994). AHR belongs to the basic helix-loop-helix/Per-Arnt-Sim (bHLH/PAS) family and

translocates into the nucleus and heterodimerizes with another bHLH/PAS protein, aryl hydrocarbon nuclear translocator (ARNT) (Crews 1998). This AHR/ARNT complex binds to specific enhancer sequences adjacent to target promoters termed xenobiotic responsive elements (XREs) and enhances the transcription of target genes encoding phase 1 metabolic enzymes such as the cytochrome P450 family (CYP) 1A1 and CYP1A2, phase 2 metabolic enzymes such as uridine diphosphate glucuronosyltransferase (UGT) 1A1 and UGT1A6, and phase 3 metabolic enzymes such as the ATP-binding cassette (ABC) transporter, subfamily G, member 2 (ABCG2) (Whitlock 1999; Nakata et al. 2006). Therefore, AHR was originally regarded as a key regulator of xenobiotic metabolism.

Recently, many studies have reported that AHR may have alternate biological roles in addition to its regulation of xenobiotic metabolism. AHR functions as a ligand-dependent E3 ubiquitin ligase of certain nuclear receptors (Ohtake et al. 2007). Kawajiri et al. (2009) reported that AHR played a critical role in the suppression of intestinal carcinogenesis by a previously undescribed ligand-dependent mechanism of proteasomal β -catenin degradation and the natural AHR ligands of indole derivatives such as indole-3-carbinol and 3,3-diindolylmethane suppressed intestinal carcinogenesis in an AHR-dependent manner. More recently, there have been some reports that AHR plays an important role in the modulation of the intestinal immune system (Hooper 2011). Some reports have indicated that AHR regulates regulatory T cell (Treg) and T helper 17 (Th17) cell differentiation in gut lamina propria and mesenteric lymph nodes in a ligand-specific manner (Singh et al. 2011). It was also reported that AHR controls the maintenance and differentiation of intestinal lymphoid cells (Kiss et al. 2011; Li et al. 2011). These reports suggest that AHR not only plays a significant role in the regulation of xenobiotic metabolism, but also in the prevention of intestinal carcinogenesis and modulation of gut immunity.

The prototypical AHR ligands are the known environmental contaminants 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), polycyclic aromatic hydrocarbons (PAHs), and halogenated aromatic hydrocarbons (HAHs) (Fernandez-Salguero et al. 1996; Sogawa and Fujii-Kuriyama 1997). Moreover, some dietary compounds have the potential to be AHR ligands. In previous studies, flavonoids (Amakura et al. 2003; Zhang et al. 2003; Wang et al. 2012), indole compounds (Heath-Pagliuso et al. 1998; Degner

et al. 2009), and curcumin (Ciolino et al. 1998) were shown to act as natural AHR ligands. An assay system to effectively evaluate AHR-mediated transcriptional activity is needed because a variety of drugs, environmental pollutants, and food nutrients control the physiological processes mediated by AHR.

The aim of this study was to establish a stable cell line for screening of AHR-activating compounds. Until now, AHR-mediated transcriptional activity represented by the transcriptional activity of CYP1A1, whose promoter contains XRE, has been applied as a screening procedure for AHR-activating compounds (Garrison et al. 1996; Natsume et al. 2005; Hamada et al. 2006). However, the AHR-mediated transcriptional activity does not necessarily correspond to the transcriptional activity of CYP1A1 (Aix et al. 1994; Denison et al. 1998).

In order to evaluate the AHR-mediated transcriptional activity more specifically, we established a stable AHR-responsive HepG2 cell line by co-transfection of an AHR expression vector (pcDNA-hAHR) and an AHR-responsive vector (pGL3-XRE) containing three tandemly arranged XRE elements upstream of the luciferase gene into a human hepatoma derived cell line: HepG2. The cell line could be used to evaluate AHR-mediated transcriptional activity by the luciferase assay, but also to study AHR target gene expression at the mRNA level. The utility of the established stable AHR-responsive HepG2 cell line was confirmed in assays of AHR-activating compounds.

Materials and methods

Cell culture

The HepG2 cell line (derived from human hepatic cancer tissue) was obtained from the American Type Culture Collection (ATCCATCC, Manassas, VA, USA). HepG2 cells were grown and maintained at 37 °C in a Dulbecco's modified Eagle's medium (DMEM, Wako Pure Chemical Industries, Osaka, Japan) containing 10 % fetal bovine serum (FBS, Asahi Technoglass, Chiba, Japan), 4 mM L-glutamine, 40 U/mL of penicillin, and 40 μ g/mL of streptomycin under a humidified atmosphere containing 5 % CO₂. For culturing of the stable AHR-responsive HepG2 cells, 500 μ g/mL of the neomycin analog G418 sulfate (Nacalai Tesque, Kyoto, Japan) was added to the same medium as that used for culturing the HepG2 cells.

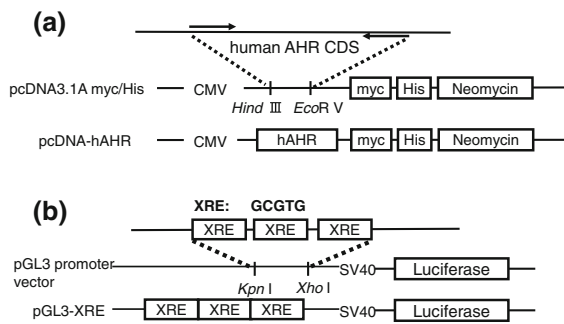


Fig. 1 Construction of the AHR expression vector and the AHR-responsive vector. **a** AHR expression vector (pcDNA-hAHR); **b** AHR-responsive vector (pGL3-XRE)

Expression vector and reporter vector construction

The AHR expression vector (pcDNA-hAHR, Fig. 1a) was constructed as follows: the open reading frame of human AHR was amplified from HepG2 cells by using the primer 5-ATAAGCTTTG GGCACCATGA ACAGC-3, which introduced an *EcoRV* site, and the primer 5-CCGATATCTC AGGAATCCAC TGGAT GTC-3, which introduced a *HindIII* site. *EcoRV/HindIII*-digested PCR fragments were ligated into appropriately digested pcDNA3.1/myc-His A (Invitrogen, Carlsbad, CA, USA). The AHR-responsive vector (pGL3-XRE, Fig. 1b) was constructed by ligating the annealed oligonucleotides, which contained three tandemly arranged XREs (GCGTG), into the *KpnI/XhoI*-digested pGL3 promoter vector (Promega, Madison, WI, USA): sense, 5-CAAGGTAC CAGTGCCTGTCAGTGCCTGTCAGTGCCTGTC TCGAGGCT-3, and antisense, 5-AGCCTCGAGA CACGCACTGACACGCACTGACACGCACTGG TACCTTG-3.

Stable integration and selection of G418-resistant colonies

The AHR expression vector and the AHR-responsive vector were stably transfected into HepG2 cells as described previously (Natsume et al. 2005). HepG2 cells were transfected with the expression vector and reporter vector by a lipofectamine technique with lipofectamine and PLUS reagent (Gibco, Gaithersburg, MD, USA). One day before transfection, 2×10^5 cells were plated on 60-mm dishes and allowed to reach 50–60 % confluency prior to replacing the medium in the dishes with a serum-free medium 1 h before

transfection. Plasmid DNA (2 μ g) and the PLUS reagent (8 μ L) were diluted in 250 μ L of the serum-free medium and then incubated at room temperature for 15 min. Lipofectamine (12 μ L) was diluted in 250 μ L of the serum-free medium. The plasmid and lipid dilutions were combined, gently mixed, and then incubated at room temperature for 15 min. Meanwhile, the medium in the dishes was replaced with 2 mL of serum-free medium. A 500- μ L portion of the DNA/lipid suspension was added to each dish and gently mixed.

The transfection process was followed by neomycin selection to ensure stable integration of plasmid DNA. After incubating for 3 h, the serum-free medium in the dishes was replaced with normal culture medium. The cells were cultured for 2 weeks in a medium containing 500 μ g/mL of G418 disulfate until colonies appeared. The G418-resistant colonies were selected and expanded in a 24-well cell culture plate (Corning-Costar Japan, Tokyo, Japan). Among these, the colonies that showed adequate number of cells to pass to 48 well plates and conduct luciferase assay (more than 50 % confluent/well) were tested for their luciferase activity by treating with 3-methylcholanthrene (3MC, Wako Pure Chemical Industries). The colony that exhibited the greatest increase in luciferase activity by treatment with 3MC was selected as the stable AHR-responsive HepG2 cell line.

Luciferase assay

The luciferase assay was carried out to assess AHR-mediated transcriptional activity as previously described (Hamada et al. 2006). The stable AHR-responsive HepG2 cells were seeded in a 24-well cell culture plate at a density of 1×10^5 cells/well. The 24-well cell culture plate had been pre-coated with Type I-C collagen (Nitta Geratin, Osaka, Japan) for 30 min before the cells were seeded. After an overnight incubation, the medium was replaced with the medium containing test samples. Each test sample was diluted in the cell culture medium at the test concentrations, with the concentration of DMSO never exceeding 0.1 % (v/v) in the cell culture medium. After 24 h of culture, the cells were washed twice with 500 μ L of phosphate-buffered saline (PBS), lysed with 100 μ L of $1 \times$ passive lysis buffer (Promega), the lysate was centrifuged at $15,000 \times g$ for 5 min, and the supernatant was collected for the luciferase assay. Luciferase

Table 1 Primers used for Real-Time PCR analysis

Gene	GenBank ID	Sequence (5' → 3')	Tm	
β-actin	NM_001101	Forward	TGGCACCCAGCACAATGAA	64.7
		Reverse	CTAAGTCATAGTCCGCTAGAAGCA	62.3
CYP1A1	NM_000499	Forward	AGATGGTCAAGGAGCACTACA	69.0
		Reverse	CTGGACATTGGCGTTCTCAT	68.0
UGT1A1	NM_000463	Forward	TGGCTGTCCCCTACTGAC	64.6
		Reverse	AGGGTCCGTCAGCATGACATC	64.7
ABCG2	NM_001257386	Forward	TGCCCAGGACTCAATGCAAC	64.5
		Reverse	TCGATGCCCTGCTTTACCAATA	64.7

activity was determined by a dual-luciferase reporter assay (Promega) with an LB9507 Lumet luminometer (Berthold Technologies, Bad Wildbad, Germany).

Chemicals

Benzo[a]pyrene (BEN), indigo (IND), 2-(1H-Indol-3-ylcarbonyl)-4-thiazolecarboxylic acid methyl ester (ITE), and 6-formylindolo[3,2-b] carbazole (FICZ) were obtained from Wako Pure Chemical Industries. These chemicals were previously established as candidate AHR ligands (Nguyen and Bradfield 2008). Each phytochemical was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 10 mM before use.

Isolation of total RNA and real-time PCR

The mRNA expression levels of CYP1A1, UGT1A1, and ABCG2 were evaluated by real-time PCR. These genes were representative drug metabolizing genes those expressions were regulated by AHR (Whitlock 1999; Nakata et al. 2006). The stable AHR-responsive HepG2 cells were seeded onto a pre-coated 24-well cell culture plate at a density of 1×10^5 cells/well. After an overnight incubation, the medium was replaced with a medium containing each test sample. After 24 h of culture, total RNA was extracted from the cells using TRIzol Reagent (Invitrogen). cDNA was prepared from 1 µg of the total RNA. Real-time PCR was performed with SYBR Premix Ex Taq II (TAKARA BIO INC, Otsu, Japan) and 7900HT Fast Real-Time PCR System (Applied Biosystems, Tokyo, Japan). After denaturing at 95 °C for 30 s, PCR was performed for 40 cycles, each of which consisted of denaturing at 95 °C for 5 s, annealing at 60 °C for

30 s, and dissociating at 95 °C for 15 s, 60 °C for 60 s, and 95 °C for 15 s. The PCR primers used for CYP1A1 and UGT1A1 were synthesized at Invitrogen, and those used for β-actin and ABCG2 were synthesized at TAKARA BIO INC. The sequence of each primer is shown in Table 1. The mRNA levels of AHR-target genes were normalized to those of β-actin mRNA.

Statistical analysis

The data were expressed as the mean ± SD. Time-dependent and dose-dependent effects were analyzed using Tukey multiple comparison test with one-way

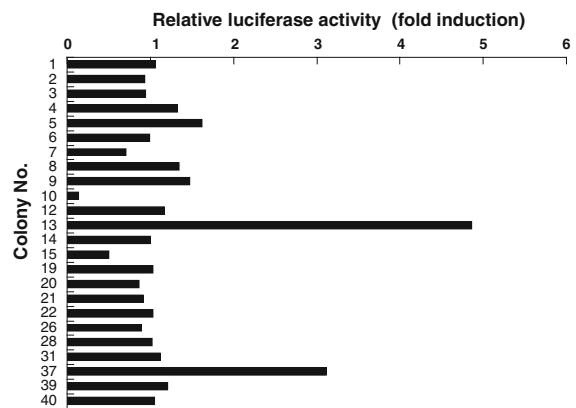


Fig. 2 Effect of 3MC on AHR-mediated transcriptional activity in G418-resistant colonies. Each colony was seeded in a collagen-coated 24-well cell culture plate at a density of 1×10^5 cells/well. After an overnight incubation, the cells were incubated with a medium containing 3MC at 5 µM or 0.05 % DMSO (control) for 24 h, and the luciferase assay was performed as described in “Materials and Methods”. The data are represented as a ratio to the control. Each value is the mean ($n = 2$)

ANOVA. Statistically significant differences between the control group and each chemically-treated group were determined by Dunnett's test. The relationship between the induction of AHR-mediated transcriptional activity and AHR-target gene expression was analyzed using the Pearson product-moment correlation coefficient.

Results

Construction of a stable AHR-responsive HepG2 cell line

Forty-eight G418-resistant colonies (colony no. 1–48) were selected, and 24 colonies that showed adequate cell growth were tested for their luciferase activity. Three colonies (colony no. 5, 13, and 37) exhibited substantially increased luciferase activity with 3MC treatment, showing more than a 1.5-fold induction (Fig. 2). Furthermore, in colonies 13 and 37, 3MC

increased the luciferase activity in a concentration-dependent manner, with the highest increase in luciferase activity being observed in colony 13 (Fig. 3b, c). Thus, colony 13 was selected as the stable AHR-responsive HepG2 cell line.

Time- and dose-dependent changes in the AHR-mediated transcriptional activity in stably transfected HepG2 cells after 3MC treatment

The luciferase activity increased in a time-dependent manner up to 24 h after 3MC treatment and gradually decreased thereafter (Fig. 4a). Therefore, the reaction time was fixed at 24 h. The effect of various concentrations of 3MC on the induction of luciferase was further examined. 3MC-mediated increases in luciferase activity occurred in a concentration-dependent manner up to 2.5 μM and were maintained for up to 10 μM (Fig. 4b).

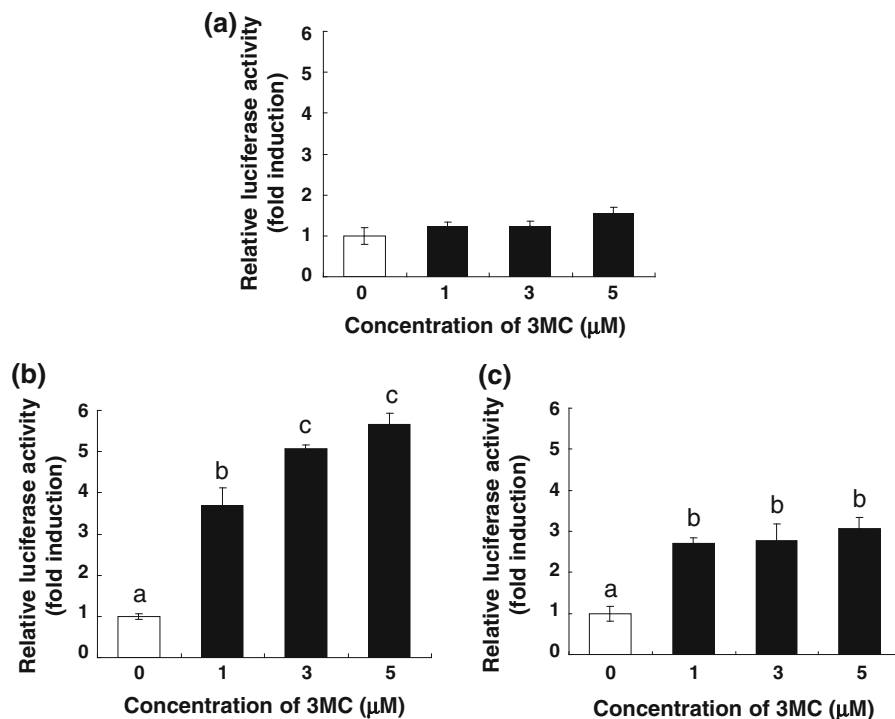


Fig. 3 Dose dependence of the increase in AHR-mediated transcriptional activity following 3MC treatment in colony 5 (a), 13 (b), and 37 (c). Each colony was seeded in a collagen-coated 24-well cell culture plate at a density of 1×10^5 cells/well. After an overnight incubation, the cells were incubated with a medium containing 3MC at 1, 3, and 5 μM or 0.05 % DMSO (control) for 24 h, and the luciferase assay was

performed as described in “Materials and Methods”. The data are represented as a ratio relative to the corresponding controls. Each value is the mean \pm SD ($n = 3$). Dose-dependent effects were analyzed using Tukey multiple comparison test with one-way ANOVA. Points not sharing a common letter are significantly different ($p < 0.05$)

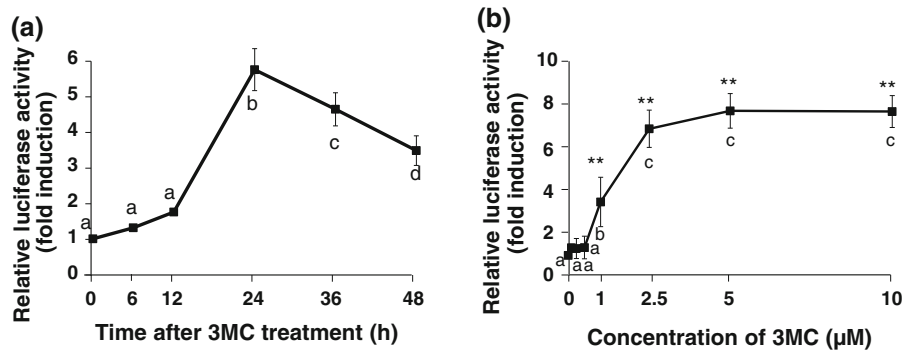


Fig. 4 Time-course or dose-dependent changes in the AHR-mediated transcriptional activity in stably transfected HepG2 cells after treatment with 3MC. The cells were treated with 3MC (2.5 μM) or with 0.025 % DMSO (control) for the indicated times (a) or were treated with 3MC at the indicated concentrations or 0.1 % DMSO (control) for 24 h (b), and total cell lysates were used for the luciferase assay. Luciferase activity

was measured as described in “Materials and Methods”, and the data are represented as a ratio relative to the corresponding controls. Each value is the mean \pm SD ($n = 3$). Time- and dose-dependent effects were analyzed using Tukey multiple comparison test with one-way ANOVA. Points not sharing a common letter are significantly different ($p < 0.05$)

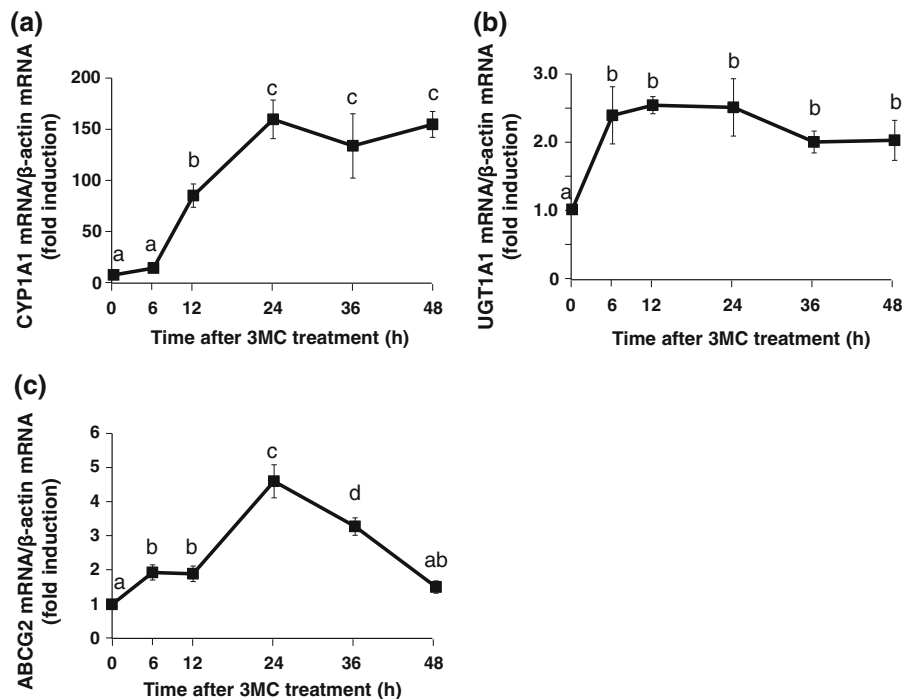


Fig. 5 Time-course changes in the mRNA level of AHR-target genes in the stably transfected HepG2 cells after treatment with 3MC. The cells were treated with 3MC (2.5 μM) or with 0.025 % DMSO (control) for the indicated times. Total RNA from the cells was used for real-time RT-PCR analysis, and mRNA expression levels of CYP1A1 (a), UGT1A1 (b), and ABCG2 (c) were evaluated. The mRNA levels of AHR-target

genes were normalized to those of β-actin mRNA and represented as a ratio relative to the corresponding controls. Each value is the mean \pm SD ($n = 3$). Time-dependent effects were analyzed using Tukey multiple comparison test with one-way ANOVA. Points not sharing a common letter are significantly different ($p < 0.05$)

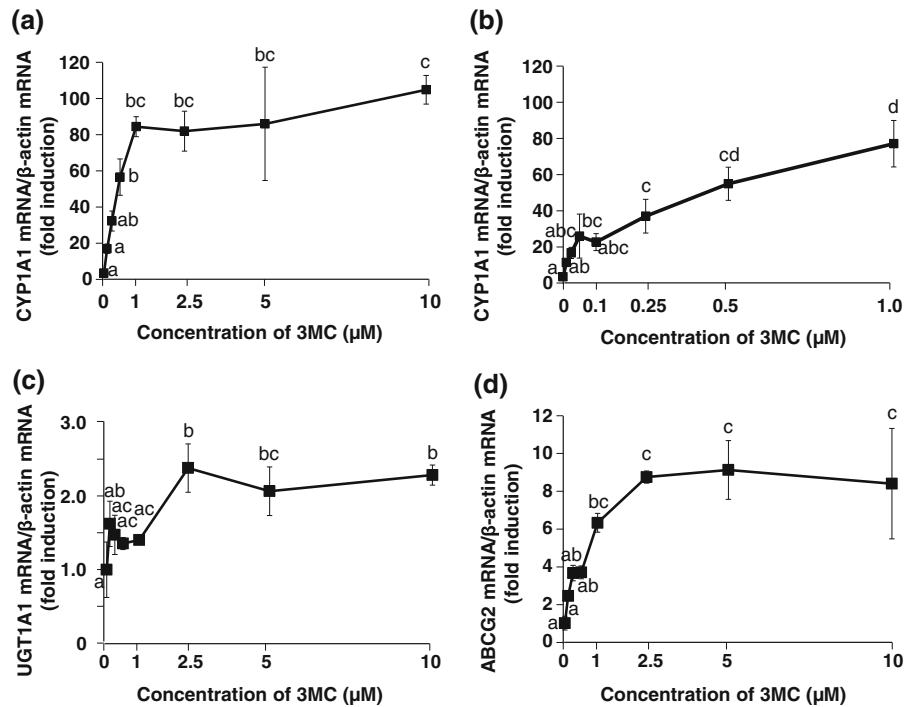


Fig. 6 Dose-dependent changes in the mRNA level of AHR-target genes in the stably transfected HepG2 cells after treatment with 3MC. The cells were treated with 3MC at the indicated concentrations or 0.1 % DMSO (control) for 24 h. Total RNA from the cells was used for real-time RT-PCR analysis, and mRNA expression levels of CYP1A1 (treated with 0–10 μ M 3MC) (a), CYP1A1 (treated with 0–1 μ M 3MC) (b), UGT1A1

(c), and ABCG2 (d) were evaluated. The mRNA levels of AHR-target genes were normalized to those of β -actin mRNA and represented as a ratio relative to the corresponding controls. Each value is the mean \pm SD ($n = 3$). Dose-dependent effects were analyzed using Tukey multiple comparison test with one-way ANOVA. Points not sharing a common letter are significantly different ($p < 0.05$)

Time-course changes in the mRNA level of the AHR-target genes in stably transfected HepG2 cells after 3MC treatment

CYP1A1, UGT1A1 and ABCG 2 were known as representative target genes of AHR (Whitlock 1999; Nakata et al. 2006). We detected the mRNA expression of these genes to estimate AHR transcriptional activity. The CYP1A1 mRNA levels increased in a time-dependent manner up to 24 h, and this increase was maintained for up to 48 h (Fig. 5a). The expression of UGT1A1 mRNA was maximal at 6 h and was maintained for up to 48 h (Fig. 5b). The ABCG2 mRNA level increased in a time-dependent manner up to 24 h and thereafter gradually decreased (Fig. 5c). Based on these results, the reaction time was fixed at 24 h.

Dose-dependent changes in the mRNA level of AHR-target genes in stably transfected HepG2 cells after treatment with 3MC

CYP1A1 expression increased in a concentration-dependent manner up to 1 μ M (Fig. 6a, b) and maintained for up to 10 μ M. Moreover, there was a significant positive correlation between relative luciferase activity and the gene expression of CYP1A1, and the correlation was strong even when the concentration of 3MC was lower than 1 μ M (Fig. 7a, b). The 3MC-mediated increase in UGT1A1 mRNA expression was saturated at 2.5- μ M 3MC treatment and was maintained for up to 10 μ M (Fig. 6c). There was a significant positive correlation between relative luciferase activity and the expression of UGT1A1 (Fig. 7c). ABCG2 expression was increased in a

Fig. 7 Correlations between luciferase activities and the levels of CYP1A1 (treated with 3MC at 0.1–10 μ M) (a), CYP1A1 (treated with 3MC at 0.01–1 μ M) (b), UGT1A1 (c), and ABCG2 (d) mRNA after the 3MC-treatment. The relationship between relative luciferase activities and the level of each mRNA was analyzed using the Pearson product-moment correlation coefficient

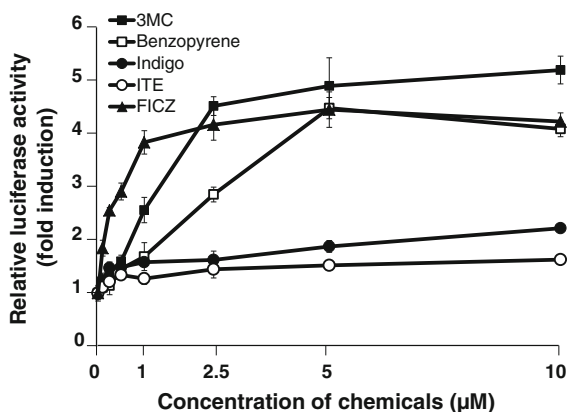
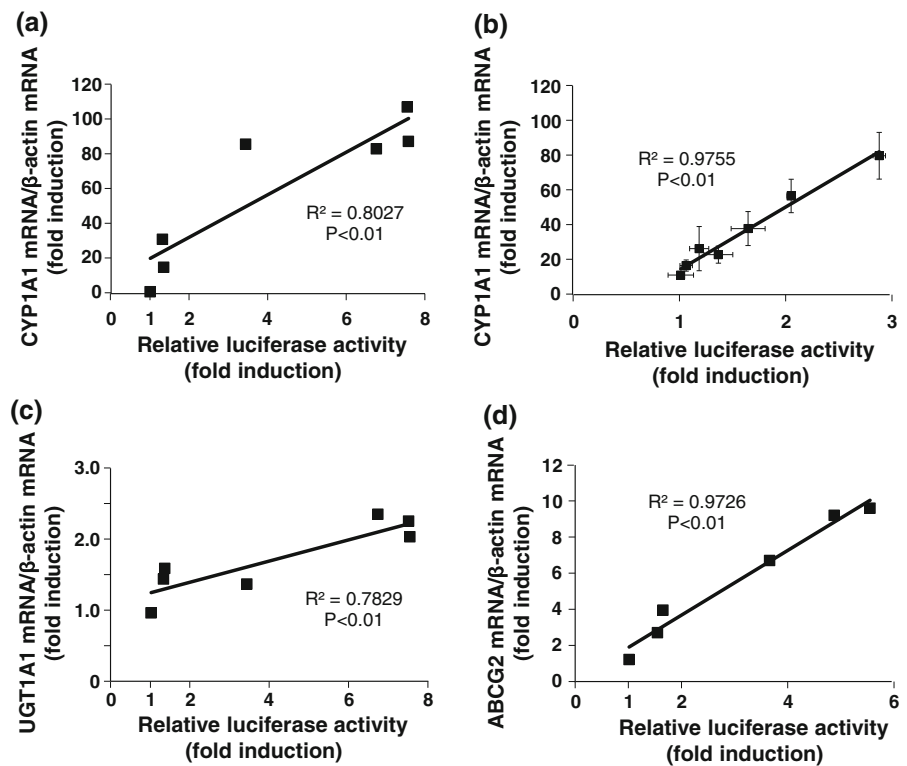


Fig. 8 Effects of various chemicals on the AHR-mediated transcriptional activity in stably transfected HepG2 cells. The stable AHR-responsive HepG2 cells were treated with each chemical at the indicated concentrations or 0.1 % DMSO (control) for 24 h, and the luciferase assay was performed as described in “Materials and Methods”

concentration-dependent manner up to 2.5 μ M and was maintained for up to 10 μ M (Fig. 6d). There was a significant and strong positive correlation between the relative luciferase activity and the gene expression of ABCG2 (Fig. 7d).

Effects of various chemicals on the induction of AHR-mediated transcriptional activity in the stably transfected HepG2 cells

In this experiment, 3MC, BEN, IND, ITE, and FICZ were used as representative AHR ligands (Nguyen and Bradfield 2008). Treatment of the stable AHR-responsive HepG2 cells with representative AHR ligands for 24 h resulted in significant increases in luciferase activity in a dose-dependent manner (Fig. 8). 3MC, BEN, and FICZ all induced greater luciferase activity than IND and ITE. The AHR-mediated transcriptional activity following FICZ-treatment reached saturation at a lower concentration (1 μ M) compared to 3MC and BEN treatment (Fig. 8).

Effects of various chemicals on the induction of AHR-target genes in the stably transfected HepG2 cells 3MC, BEN, and FICZ all induced greater the mRNA expression of AHR-target genes than IND and ITE (Fig. 9a, b, c). This is the same pattern as that obtained for luciferase activity. Relationship analyses revealed a significant and positive correlation between the relative luciferase activity and AHR-target gene expression (Fig. 10a, b, c).

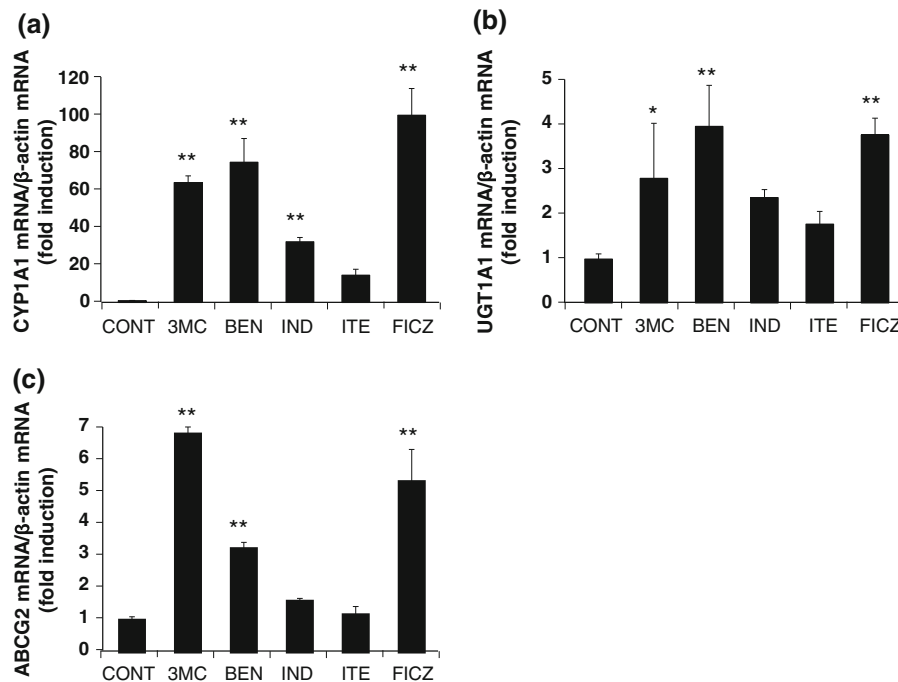


Fig. 9 Effects of various chemicals on the mRNA level of AHR-target genes in stably transfected HepG2 cells. The stable AHR-responsive HepG2 cells were treated with each chemical (5 μ M) or 0.05 % DMSO (control) for 24 h and total RNA from the cells was used for real-time RT-PCR analysis. The levels of

CYP1A1 (a), UGT1A1 (b), and ABCG2 (c) mRNA were normalized to that of β -actin mRNA and represented as a ratio relative to the corresponding controls. Significant differences from the corresponding controls assayed were determined by Dunnett's test; * $p < 0.05$, ** $p < 0.01$

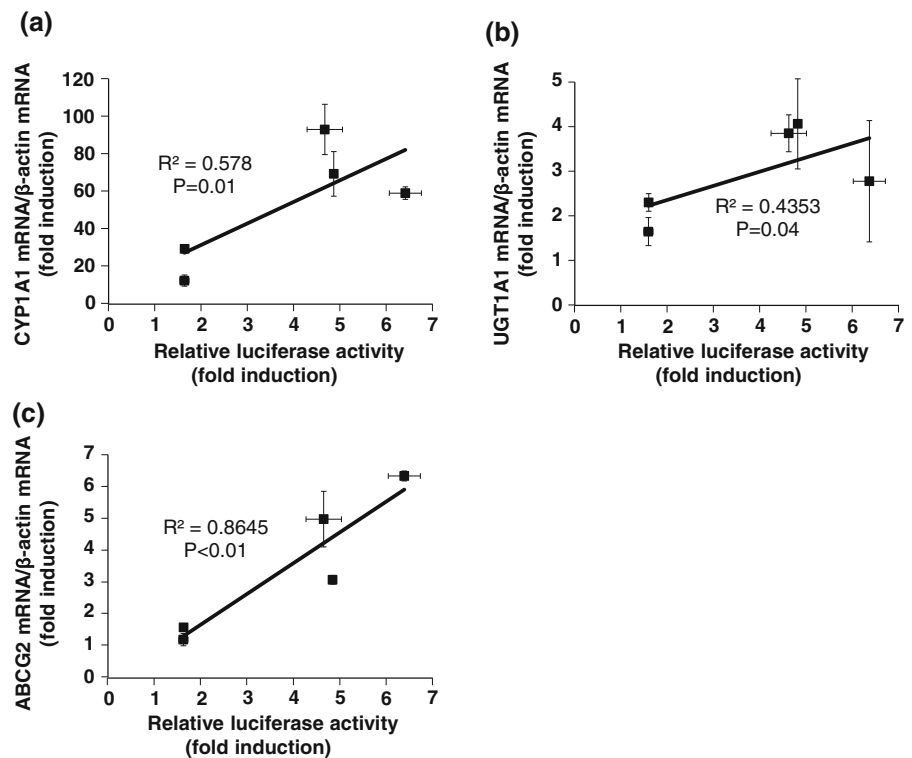
Discussion

In this study, we established the stable AHR-responsive HepG2 cell line by co-transfection of the AHR-responsive vector (pGL3-XRE) and the AHR expression vector (pcDNA-hAHR) into HepG2 cells, and AHR-mediated transcriptional activity in this cell line (based on the luciferase assay) was assessed using 3MC, a representative AHR activator. The time- and concentration-dependent induction of luciferase activity by 3MC was clearly observed in the stable AHR-responsive HepG2 cell line. These effects were comparable to the results obtained using the method for evaluating the CYP1A1 transcriptional activity (Garrison et al. 1996; Fazili et al. 2010). The difference between transcriptional activity of CYP1A1 and AHR-mediated transcriptional activity is not marked when treated with strong AHR-activating compounds such as 3MC. However, CYP1A1 expression is regulated by transcriptional factors other than AHR, including the constitutive androstane receptor (CAR), peroxisome proliferator-activated receptor (PPAR)- α , and the liver

X receptor (LXR) (Kaczynski et al. 2002, S  r  e et al. 2004, Yoshinari et al. 2010, Shibahara et al. 2011). Therefore, the difference between CYP1A1 transcriptional activity and AHR-mediated transcriptional activity is more considerable when the treatment compounds activate transcriptional factors other than AHR.

Time- and concentration-dependent inductions of AHR target gene transcription by 3MC were observed in the stable AHR-responsive HepG2 cell line, and significant positive correlations between relative luciferase activity and mRNA expression of AHR-target genes were also observed. In previous reports, 3MC induced the gene expression of CYP1A1 (Fazili et al. 2010; Tsuchiya et al. 2003; Stejskalova et al. 2011), UGT1A1 (Smith et al. 2005), and ABCG2 (Tompkins et al. 2010) in many types of cultured tissues and cells. Moreover, it has been reported that 3MC most strongly induces CYP1A1 expression among the three AHR-target genes, CYP1A1, UGT1A1, and ABCG2 (Stejskalova et al. 2011). These reports correspond to the results in this study. There was a significant positive

Fig. 10 Correlations between relative luciferase activities and the levels of CYP1A1 (a), UGT1A1 (b), and ABCG2 (c) mRNA after chemical treatment. The relationship between relative luciferase activities and the level of each mRNA was analyzed using the Pearson product-moment correlation coefficient



correlation between the induction of AHR-mediated transcriptional activity and CYP1A1, UGT1A1, and ABCG2 mRNA expression (Fig. 7). The correlation between the induction of CYP1A1 mRNA expression and AHR-mediated transcriptional activity is particularly strong in a low concentration range (0.01–1.0 μM). Multiple XREs are located 1,252–892 bp upstream of the CYP1A1 promoter and play an important biological role in regulating CYP1A1 expression (Fujii-Kuriyama et al. 1992; Garrison et al. 1996; Fazili et al. 2010). Conversely, XREs are located approximately 3,424–3,299 bp upstream of the UGT1A1 promoter and 2,357–2,333 bp upstream of the ABCG2 promoter (Yueh et al. 2003; Tompkins et al. 2010). This difference in the location and number of XREs may be responsible for the divergent effects of 3MC on AHR-target gene expression.

Luciferase activity in the stable AHR-responsive HepG2 cell line was increased not only by 3MC, but also by the other known AHR ligands BEN, IND, ITE, and FICZ. This suggests that the stable AHR-responsive HepG2 cell line can be used for the evaluation of AHR-mediated transcriptional activity of AHR-activating compounds. Induction of AHR-mediated

transcriptional activity by FICZ plateaued at a lower concentration (1 μM) than the other AHR activators (Fig. 8). The affinity of FICZ for AHR is higher than that of the other AHR agonists used in this study (Nguyen and Bradfield 2008). This characteristic of FICZ may be the reason why the stable AHR-responsive HepG2 cell line responds to low concentrations of FICZ. There was a significant and positive correlation between relative luciferase activity and the gene expression of AHR-target genes (Fig. 10a, b, c). This suggests that the stable AHR-responsive HepG2 cell line can be used for evaluation of AHR-mediated transcriptional activity.

In conclusion, we have established a stable AHR-responsive HepG2 cell line, which is the useful tool for the screening of the AHR-activating compounds. Studies on the induction of AHR-mediated transcriptional activity by drugs or food nutrients are important for understanding the effects of these modulators, because the activation of AHR is associated with multiple physiological functions. Therefore, the established stable AHR-responsive HepG2 cell line will greatly contribute to the understanding of the role of AHR-activating compounds in human health.

Acknowledgments This work was supported by a Grant-in-Aid for Young Scientists (B-22780115) from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) of Japan.

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