HISTONE DEACETYLASES ARE INVOLVED IN SPECIES-SPECIFIC MODULATION OF ARYLHYDROCARBON RECEPTOR-DEPENDENT GENE EXPRESSION IN HUMANS AND MICE

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Abstract

The arylhydrocarbon receptor (AhR) mediates toxicities of dioxins, including the most potent congener 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), by being translocated to the nucleus upon ligand-binding and inducing expression of target genes. Although the species-specific activity of the AhR is primarily attributable to species-specific AhR-ligand affinity, the precise mechanism has not been clarified. We investigated the modulation mechanisms of AhR in Hepa1c1c7 and HepG2 hepatoma cells, which were derived from high-affinity-AhR-expressing C57BL/6 mice and low-affinity-AhR-expressing humans, respectively. Although, consistent with their AhR affinities, TCDD induced a greater amount of cytochrome P450 1A1 (CYP1A1) mRNA, one of the most sensitive AhR-targets, in Hepa1c1c7 cells than in HepG2 cells immediately after exposure, both cells expressed a similar level of CYP1A1 mRNA from 4 h onward. Treatment of cells with trichostatin A (TSA), a histone deacetylase (HDAC) inhibitor, resulted in greater *CYP1A1* induction at 4-6 h in Hepa1c1c7 cells than in HepG2 cells. ChIP assay showed that HDAC2 binding to the *CYP1A1* promoter region increased at 4-6 h after TCDD exposure in Hepa1c1c7 cells and HDAC1 binding to the *CYP1A1* promoter region decreased after TCDD exposure in HepG2 cells. These results suggest that HDACs are involved in determining the species-specific modulation mechanism of AhR function.

Introduction

The arylhydrocarbon receptor (AhR) is a ligand-activated transcription factor and mediates the toxicity of dioxins including TCDD. Upon binding to TCDD, the AhR becomes activated and exerts its effects by binding to the specific DNA sequences called XREs and induces or modulates the target gene expression, such as *CYP1A1*.[1]

While TCDD toxicity is though to be mediated by the AhR-dependent reactions described above in mammalians in common, susceptibility to TCDD toxicity varies greatly among animal species and strains.[2] The major cause of the species- and strain-specific differences in susceptibility has primarily been attributed to differences in the affinity of the AhR for the ligand.[3] However, our recent study suggested the existence of critical factors besides AhR affinity that modulate the expression of the target genes by AhR activation.[4] Previous studies using ligation-mediated PCR and recent studies by chromatin immunoprecipitation (ChIP) assay have indicated the involvement of histone acetylation and remodeling of chromatin structure in the course of AhR-dependent gene expression.[5,6,7] Although HDACs are the chromatin-remodeling factors and repress gene expression by deacetylating histone,[8,9] the contribution of the differences in these properties to the species-specific gene expression by the AhR in human and mouse cells has not been addressed.

In the present study we investigated the AhR activation pathway upon TCDD treatment in Hepa1c1c7 cells, established from C57BL/6 mice which express high-affinity AhR, and HepG2 cells, established from humans which express low-affinity AhR, in order to explore the factors involved in the species-specific regulation of

AhR-dependent gene expression. We compared time-course profiles of CYP1A1 mRNA induction by real-time PCR and recruitment of the HDACs to the *CYP1A1* promoter region by ChIP assay in the two representative human and mouse hepatoma cell lines.

Materials and Methods

Reagents and Cells

TCDD (purity 98%) was purchased from Cambridge Isotope Laboratory. Trichostatin A (TSA) were purchased from Wako and Sigma, respectively. Hepa1c1c7 and HepG2 cells were maintained at 37 °C in DMEM supplemented with 10% FBS, 100 units/ml penicillin, and 100 μg/ml streptomycin under a humidified atmosphere composed of 95% air and 5% CO_2.

RT-PCR

Total RNA was isolated from cells with an RNeasy Mini kit. After checking the quality of the RNA by electrophoresis, reverse transcription was performed with an RNA LA PCR kit ver3.0 using 100 ng of total RNA as described previously.[4]

Real-time PCR

Quantitative real-time PCR analysis was performed on a LightCycler instrument as described previously.[4] The relative mRNA level per cell was determined by dividing the amount of amplicons obtained by real-time PCR of 100 ng total RNA by the cell number that represents 100 ng total RNA.

Western blotting

Nuclear and cytoplasmic fractions were prepared using NE-PER Nuclear and Cytoplasmic Extraction Reagent. Western blotting was carried out as described previously[10] with a minor modification. The antibodies used for western blotting were anti-HDAC1, anti-HDAC2, or anti-HDAC3.

Chromatin immunoprecipitation (ChIP) assay

ChIP assay carried out as described previously[6] with a minor modification. The antibodies used for ChIP assay were anti-AhR, anti-RNA polymerase II, anti-HDAC1, anti-HDAC2 or anti-HDAC3. DNA fragments were suspended in 10 μl of TE buffer and a 1 μl sample was subjected to real-time PCR.

Results and Discussion

Characteristics of the time course of CYP1A1 induction by TCDD in Hepa1c1c7 cells and HepG2 cells

The time course of *CYP1A1* induction by TCDD was measured in both cell lines by real-time PCR using the primers designed in our previous study for regions that were 100% homologous in mice and humans so as to achieve similar PCR efficiency in both species.[4] Because of the much higher AhR affinity for TCDD in C57BL/6 mice than in humans, *CYP1A1* induction was expected to be more intense in the Hepa1c1c7 cells than in the HepG2 cells, and as expected 2 h after exposure to 10 nM TCDD more CYP1A1 mRNA was detected in the Hepa1c1c7 cells than in HepG2 cells (Fig. 1A). From 4 h onward, however, *CYP1A1* induction in HepG2 cells was similar to or rather higher than that in Hepa1c1c7 cells, which did not reflect the difference in affinity of their AhR for TCDD.

HDACs are involved in the difference of the CYP1A1 induction between Hepa1c1c7 cells and HepG2 cells

Recent studies have indicated the involvement of remodeling of chromatin structure in the course of AhR-dependent gene expression.[6,7] HDACs are the chromatin-remodeling factors and repress gene expression by deacetylating histone.[8,9] TSA is one of HDAC inhibitors, and TSA treatment may increase the acetylation of histone H4 in the region of *CYP1A1* gene, so that the activated AhR can easily access to XRE and can activate transcription of the *CYP1A1* gene. To investigate the influence of HDACs on the *CYP1A1* gene expression, which did not reflect their AhR affinity from 4 h onward, in Hepa1c1c7 cells and HepG2 cells, we measured time-course of *CYP1A1* induction in both cell lines treated with TCDD and TSA. Treatment of cells with the TSA resulted in greater *CYP1A1* induction at 4 h and 6 h in Hepa1c1c7 cells than in HepG2 cells (Fig. 1B), in contrast to the same levels of *CYP1A1* induction in both cells after 4 h in the absence of TSA (Fig. 1A). Since the cells started to detach from the dishes after 12 h of culture in the presence of TSA, it seemed impossible to accurately compare the *CYP1A1* induction profile in the two cell lines thereafter. These results suggested that HDACs are involved in the cause of the *CYP1A1* induction, which did not reflect their AhR affinity from 4 h onward, in Hepa1c1c7 cells and HepG2 cells.

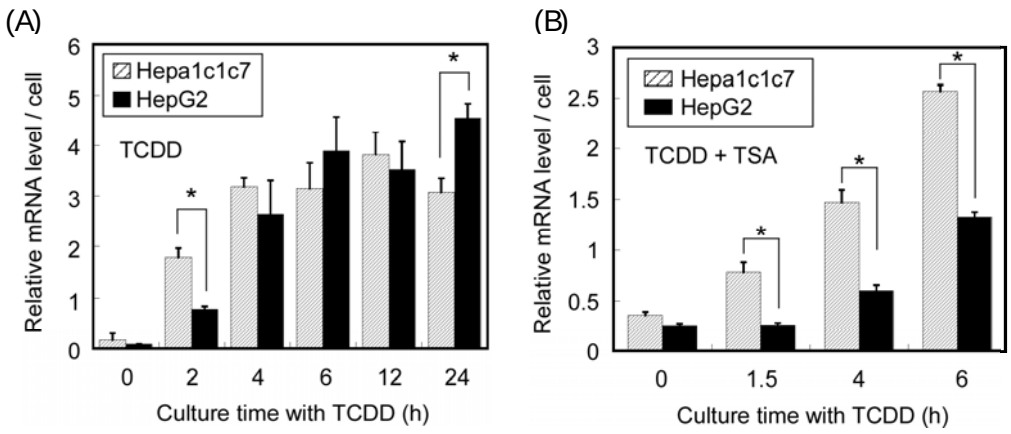

Fig. 1, Comparison between the time course of *CYP1A1* induction by (A) TCDD or (B) TCDD and TSA in Hepa1c1c7 cells and HepG2 cells. The data are expressed as means ± S.E. * Statistically significant difference between Hepa1c1c7 and HepG2 at $P < 0.05$..

Comparison of protein expression of Class I HDACs in Hepa1c1c7 cells and HepG2 cells

Class I HDACs, HDAC1, -2, -3, and -8, are expressed ubiquitously in organs including the liver. Thus, we investigated the expression of mRNA and protein of Class I HDACs in Hepa1c1c7 cells and HepG2 cells in order to determine how HDACs are involved in *CYP1A1* induction. The expression of HDAC1, HDAC2 and HDAC3 mRNA were at similar levels one another and much higher than HDAC8 mRNA in Hepa1c1c7 cells and HepG2 cells. The protein amounts of HDAC1, HDAC2 and HDAC3 in the nucleus of Hepa1c1c7 cells were at similar levels in HepG2 cells. These results showed that the protein amounts of HDAC1, HDAC2 and HDAC3 do not account for the difference in the *CYP1A1* induction in Hepa1c1c7 cells and HepG2 cells.

Involvement of HDAC1 and HDAC2 in the binding of AhR and RNA polymerase II (PolII) to the CYP1A1 promoter region in Hepa1c1c7 cells and HepG2 cells

To investigate functional involvement of the HDACs, we measured binding of the HDAC1, HDAC2 and HDAC3 to the *CYP1A1* promoter region in Hepa1c1c7 cells and HepG2 cells by ChIP assay. In Hepa1c1c7 cells, although no binding of HDAC1 and HDAC3 to the *CYP1A1* promoter region was detected, HDAC2 binding to the *CYP1A1* promoter region peaked at 4-6 h after TCDD exposure. On the other hand, in HepG2 cells, although no binding of HDAC2 and HDAC3 to *CYP1A1* promoter region was detected, HDAC1 binding to the *CYP1A1* promoter region decreased after TCDD exposure. We also investigated binding of the AhR and PolII to the *CYP1A1* promoter region in Hepa1c1c7 cells and HepG2 cells by ChIP assay. The AhR binding and PolII recruitment to the *CYP1A1* promoter region peaked at 1.5 h after TCDD exposure in Hepa1c1c7 cells, but the AhR binding and PolII recruitment to the *CYP1A1* promoter region slowly reached a peak between 4-12 h in HepG2 cells. Thus, these results suggest that recruitment of HDAC2 to the *CYP1A1* promoter region contributes to suppression of the recruitment of AhR and PolII to the *CYP1A1* promoter in Hepa1c1c7 cells, or dissociation of HDAC1 from the *CYP1A1* promoter region contributes to the enhanced recruitment of AhR and PolII to the *CYP1A1* promoter in HepG2 cells, and results in the same levels of CYP1A1 mRNA in Hepa1c1c7 cells as in HepG2 cells.

These results of the present study demonstrated differences between the two cell lines in the kinetics of the HDAC1 and HDAC2 binding to the target gene. This has been implicated in determining the species-specific modulation mechanism of AhR function.

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