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Reliable and sensitive adenovirus-based reporter system for high-throughput screening of dioxins

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HIGHLIGHTS

- A sensitive recombinant adenovirus, Ad-6xDR, for dioxin screening is generated.
- The system is stable and exhibits superior sensitivity/responsiveness to dioxins.
- A high correlation between the virus-based reporter assay and HRGC/HRMS is shown.
- The virus is easy to perform DNA modification for improving dioxin sensitivity.

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ABSTRACT

Dioxin-responsive element (DRE)-driven luciferase reporter assay is a rapid and cost-effective screening method for detection and semi-quantitation of dioxins and dioxin-like chemicals in samples from a variety of matrices. However, most of such stable cell lines gradually lose their dioxin responsiveness over time, possibly due to cellular degradation and/or silence of the transfected reporter gene. The study aimed to establish a recombinant adenovirus-based DRE-driven luciferase reporter system for easy maintenance and improvement of dioxin sensitivity/responsiveness. Both the Ad-4xDR adenovirus-based and the Huh7-4xDRE-Luc cell-based reporter systems carry a luciferase reporter gene driven by the same 4xDRE-TATA cassette, i.e., 4 copies of DRE and a TATA box. Comparison of the two reporter systems indicated that the adenovirus-based system was much more sensitive and responsive to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) than the cell-based system. Among the four recombinant adenoviruses, Ad-4xDR, Ad-6xDR, Ad-9xDR, and Ad-15xDR carrying 4, 6, 9, and 15 copies of DRE, respectively, the Ad-6xDR-infected H4IIE cells showed the highest TCDD responsiveness. Following optimization of assay conditions, including temperature, dimethyl sulfoxide (DMSO) concentration, and multiplicity of infection (MOI) of adenovirus, the Ad-6xDR-based system exhibited markedly superior performance in TCDD sensitivity (limit of detection (LOD) = 0.02 pM) and responsiveness (the half maximal effective concentration (EC_{50}) = 10.41 pM and a maximum of 409.34 folds of luciferase induction at 300 pM TCDD). Analysis of soil samples showed a high correlation between the Ad-6xDR-based reporter assay and high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS). The study reveals that, as compared with our previous cell-based system, the Ad-6xDR-based reporter system is more reliable and sensitive for high-throughput screening of dioxins.

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1. Introduction

Halogenated aromatic hydrocarbons (HAHs), such as polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs), are a diverse group of widespread anthropogenic environmental contaminants

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(Abraham et al., 1998). These lipophilic compounds are persistent in environments and tend to accumulate via food chains. These organochlorines compounds have been found widespread in ambient air (Lee et al., 2004), stack flue gas (Wang et al., 2003), sediment (Ling et al., 1995), fish (Lee et al., 2006), blood and placentas (Wang et al., 2004), and breast milk (Chao et al., 2004, 2005). HAHs persistently contaminate our environment leading to raising public concern on human exposure to these chemicals. Therefore, it is of great importance to effectively monitor the background levels of HAHs, especially dioxins and dioxin-like compounds, in environments, foodstuffs, and human bodies.

Recent techniques for detection and quantification of dioxins and dioxin-like compounds mainly rely on costly and time-consuming high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS). To ensure environmental safety, it is extremely crucial to develop high-throughput assays for screening potential contamination of dioxin-like chemicals. We previously established a dioxin-responsive element (DRE)-driven luciferase reporter cell line, Huh7-4xDRE-Luc (Chao et al., 2006, 2007); the cells have been used in high-throughput screening of dioxin-contaminated soil, sediment, and fish samples (Chao et al., 2011, 2012). The technique requires labor-intensive selection of stable dioxin-responsive cell lines and long-time maintenance of their dioxin responsiveness. However, it has been noted that most of the stable cell lines gradually lose their dioxin responsiveness following passages for a period of time, possibly due to silence of the promoter activity (Krishnan et al., 2006).

To overcome the problem, the study aimed to establish a more reliable adenovirus-based DRE-driven luciferase reporter system. Advantages of using recombinant adenovirus include high transfection efficiency for mammalian cells and high expression of the recombinant proteins. The recombinant adenoviruses we used are replication deficient and not toxic to host cells. Adenoviruses are relatively stable and can be easily amplified in very high titer; the characteristics make viral purification, long term storage, and dioxin sensitivity/responsiveness maintenance possible. Importantly, the adenovirus system is easy to perform molecular modification for further improvement of dioxin sensitivity/responsiveness. The adenovirus-based reporter technique is a single-use assay; the cells are subjected to dioxin exposure right after infection of the adenovirus carrying a DREs-TATA cassette. Therefore, for the assay, there is no need to passage the virus-infected cells. Theoretically, passage of host cells *per se* does not influence the dioxin responsiveness.

2. Materials and methods

2.1. Reagents

Fetal bovine serum (FBS) (10091-148), calf serum (16010-159), and penicillin/streptomycin (15140-122) were purchased from Gibco/Invitrogen (Carlsbad, CA, USA). All solvents, including toluene, n-hexane, and dimethyl sulfoxide (DMSO), were pesticide residue grade from Merck (Darmstadt, Germany), Tedia (Fairfield, OH, USA), or Sigma-Aldrich (St. Louis, MO, USA). Standard solution of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), standard solutions of PCDD/Fs, including labeled compound stock, internal standard spiking, cleanup standard spiking, and US Environmental Protection Administration (EPA) Method 1613 calibration and verification solutions, were obtained from Wellington Laboratories (Ontario, Canada). Cerilliant analytical reference standard (EDF-5416), a liquid standard of seventeen 2, 3, 7, 8-substituted PCDD/F congeners, was from Cambridge Isotope Laboratories, Inc. (Cambridge, MA, USA). Rapid cleanup was performed with a CAPE-coupled activated carbon-acid silica column set (CAPE Technologies, South Portland, ME, USA). Silica gel (100–200 mesh) was purchased from Fisher (Leicestershire, England).

2.2. Cell culture

Human embryonic kidney HEK-293 cells (BCRC-60019) and rat hepatoma H4IIE cells (BCRC-60209) were purchased from Bioresource Collection and Research Center (BCRC, Hsinchu, Taiwan). HEK-293 cells were cultured in DMEM (Invitrogen, Logan, UT, USA) with 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin. H4IIE cells were cultured in MEM medium (Invitrogen) with 0.1 mM non-essential amino acids and Earle's BSS, 10% FBS, 10% calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. Unless otherwise indicated, both cells were routinely maintained at 37 °C with a humidified mixture of 5% CO_2 and 95% air.

2.3. Generation of recombinant adenoviruses carrying a DRE-driven luciferase reporter gene

Four plasmids, pGL2-4xDRE-TATA-Luc, pGL2-6xDRE-TATA-Luc, pGL2-9xDRE-TATA-Luc, and pGL2-15xDRE-TATA-Luc, were constructed by cloning a DREs-TATA cassette (carrying 4, 6, 9, and 15 copies of DRE, respectively, and a TATA box) in front of a firefly luciferase gene of pGL2-basic vector (E1641) from Promega (Madison, WI, USA). DRE was from human CYP1A1 as previously described (Chao et al., 2006). The DREs-TATA-luciferase gene-SV40 poly-adenylation site (pA) DNA fragments were isolated by digestion of the four plasmids with *KpnI*/*SalI* and then cloned into the *KpnI*/*SalI*-digested pAdTrack for generation of the pAdTrack-DREs-TATA-Luc plasmids. Following the procedure shown in Fig. 1(A), four recombinant adenoviruses (Ad-4xDR, Ad-6xDR, Ad-9xDR, and Ad-15xDR) were generated by using the AdEasy Adenoviral Vector System (Stratagene, La Jolla, CA, USA). The recombinant adenoviruses were purified and concentrated according to the manufacturer's instruction.

2.4. Preparation of soil samples

Soil samples ($n = 41$) in the present study were from the Taiwan's soil dioxins survey project between September 2011 and March 2013 (Lin et al., 2014). Dry soil samples (10 g) were extracted with an automated Soxhlet system (Extraction System B-811 LSV, BÜCHI, Switzerland). The extracted solution was evaporated to near dryness and then transferred to a CAPE-coupled carbon-acid silica column for cleanup as previously described in detail (Chao et al., 2011; Lin et al., 2014, 2013). For the PCDD/Fs analysis by HRGC/HRMS, samples were spiked with different PCDD/F internal standards before extraction and with the labeled cleanup standards before the cleanup procedure. For the DRE-driven luciferase reporter assay, the labeled standards were not included in the extraction and cleanup procedure. Following the extraction and cleanup procedure, the final extracts were evaporated to dryness and dissolved in DMSO (100 μl) for both analyses.

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