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A novel xenobiotic responsive element regulated by aryl hydrocarbon receptor is involved in the induction of BCRP/ABCG2 in LS174T cells

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ABSTRACT

Induction of the breast cancer resistance protein (BCRP/ABCG2) expression has been found in various tissues and cell-types after exposure to chemicals including 17β-estradiol, rosiglitazone, imatinib, as well as aryl hydrocarbon receptor (AhR) activators such as 2,3,7,8-tetrachlorodibenzodioxin, 3-methylcholanthrene (3MC), and omeprazole. However, the mechanism(s) underlying AhR-related induction of ABCG2 is largely unknown. Here, we demonstrate the AhR-dependent induction of ABCG2 expression in human colon adenocarcinoma LS174T cells. Importantly, a novel distal AhR-responsive element (AhRE5) located −2357/−2333 bp upstream of the ABCG2 transcriptional start site has been identified and characterized as a functional unit pivotal to 3MC-mediated induction of ABCG2. Cell-based reporter assays revealed that deletion of AhRE5 and 4 dramatically attenuated 3MC-induced activation of ABCG2 reporter activity, while further deletion of the proximal AhRE3 and 2 only moderately changed the luciferase activities. Notably, site-directed mutation of the AhRE5 in the BCRP-3.8 kb reporter construct alone resulted in approximately 80% decrease in 3MC activation of the ABCG2 promoter; additional mutation of the AhRE4 site had negligible effect on the ABCG2 promoter activity. Moreover, chromatin immunoprecipitation assays demonstrated that treatment with 3MC significantly enhanced the recruitment of AhR to the AhRE5 occupied region, and mutation of the AhRE5 site clearly dissociated AhR protein from this promoter region. Together, these data show that the novel distal AhRE5 is critical for AhR-mediated transcriptional activation of ABCG2 gene expression in LS174T cells, and it may offer new strategies for early identification of ABCG2 inducers, which would be of benefit for preventing transporter-associated drug–drug interactions.

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1. Introduction

Initially identified as a mechanism of chemotherapeutic resistance in breast cancer cells, the breast cancer resistance protein (BCRP/ABCG2), a member of the ATP-binding cassette family of transporters, is now known to play a pivotal role in the efflux transport of xenobiotics from tissues such as intestine, liver, placenta, blood-brain barrier, as well as various stem cells [1–5]. Substrates for ABCG2-mediated transport include cholesterol, hormones (17β-estradiol), vitamins, drugs (mitoxantrone, methotrexate, topotecan) and environmental chemicals, such as benzo[a]pyrene-3-sulfate, and benzo[a]pyrene-3-glucuronide [6–8]. Perturbation of the expression and function of ABCG2 often leads to profound consequences in different tissues. For instance, inhibition of ABCG2 in tumor cells increases the intracellular drug concentrations and the efficacy of chemotherapeutics; whereas inhibition of ABCG2 in liver and intestine may attenuate its defense against environmental toxicants [7]. Compared with the extensively characterized ABCG2 expression in tumor cells, relatively little is known regarding the transcriptional regulation of ABCG2 in metabolic tissues such as the liver and intestines. In the liver,

Abbreviations: AhR, aryl hydrocarbon receptor; AhRE, AhR response element; BCRP, breast cancer resistance protein; ChIP, chromatin immunoprecipitation; CLO, clofibrate; DDI, drug–drug interactions; ER, estrogen receptor; HIF-1, hypoxia-inducible factor-1; 3MC, 3-methylcholanthrene; Nrf2, nuclear factor-like 2; NR, nuclear receptor; OMP, omeprazole; PPARγ, peroxisome proliferator-activated receptor gamma; Resv, resveratrol; TGZ, troglitazone; TCDD, 2,3,7,8-tetrachlorodibenzodioxin.

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ABCG2 is located on the canalicular membrane of the hepatocyte, and is responsible for effluxing substrates out of the liver into the bile [9]. In the small intestine, ABCG2 is located on the brush border apical surface exposed to the gut lumen. Therefore, ABCG2 is strategically placed in the small intestine to be a critical determinant of oral drug absorption.

Computer-based analysis of the 5′-upstream region of ABCG2 reveals that the human ABCG2 gene lacks a canonical TATA box, yet possesses several SP1 binding sites in the proximal promoter upstream of its transcriptional start site with a CCAAT-box and several CpG islands in its downstream proximity [10]. Like other ABC transporters, up-regulation of ABCG2 seems to be mediated through a group of transcription factors, namely nuclear receptors (NRs). To date, several putative NR-binding sites have been identified in the promoter of ABCG2 gene. These elements include an estrogen responsive element (−188 to −177), three peroxisome proliferator-activated receptor gamma (PPARγ) binding sites (−3946 to −3796), and a hypoxia-inducible factor-1 (HIF-1) site (−116 to −112) [11–13]. Nonetheless, the expression of ABCG2 is also induced by a large number of xenobiotics that are not typically known to interact with estrogen receptor (ER), PPAR, or HIF-1. For example, ABCG2 was up-regulated by phenobarbital, rifampicin, and omeprazole (OMP) in human primary hepatocytes [14]; benzopyrene conjugates were reported to induce ABCG2 expression in Caco-2 cells [7]; and imatinib, a tyrosine kinase inhibitor and ABCG2 substrate, chronically but potently induced the expression of ABCG2 in Caco-2 cells [15]. Additionally, a number of cancer cells develop their drug-resistance during the course of treatment against chemotherapeutic compounds which up-regulate the expression of ABCG2 in these cells. However, the mechanisms underlying these observed inductions are largely unknown.

Recent accumulating evidence indicates that ABCG2 expression in Caco-2 and breast cancer cells can be induced in an aryl hydrocarbon receptor (AhR)-dependent fashion [7,16,17]. Prototypical activators of AhR such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and polycyclic aromatic hydrocarbons (PAHs) strongly induced the expression of ABCG2 in Caco-2 cells. Notably, transient transfection of AhR expression vector to the AhR-deficient MCF-7AHR$_{200}$ cells substantially restored the ABCG2 expression and drug-resistance in these cells [16]. Since AhR can be activated by a broad array of xenobiotics including halogenated aromatic hydrocarbons and PAHs such as TCDD and 3-methylcholanthrene (3MC), as well as clinically used drugs and endogenous chemicals such as omeprazole and bilirubin [18,19], the potential for AhR-mediated drug–drug interactions (DDIs) through alteration of ABCG2 expression is theoretically high. Nevertheless, it is not fully understood whether and how AhR regulates the expression of ABCG2 at the transcriptional level. Here, we have identified a xenobiotic responsive element that specifically binds to AhR (AhRE) and mediates transcriptional regulation of ABCG2 in human colon cancer LS174T cells. Utilizing small interfering RNA (siRNA) knockdown, site-directed mutagenesis, chromatin immunoprecipitation (ChIP), and promoter serial deletion assays, we demonstrated that the AhRE5 located at −2357/−2333 in ABCG2 promoter is critical for AhR-mediated up-regulation of ABCG2.

2. Materials and methods

2.1. Chemicals and biological reagents

Wy-14,643, clofibrate (CLF), OMP, resveratrol (Resv), troglitazone (TGZ), and 3MC were purchased from Sigma–Aldrich (St. Louis, MO). The ginkgo biloba extract (EGB 761) was from Schwabe Pharmaceuticals (Karlsruhe, Germany). The Dual-Luciferase Reporter Assay System was purchased from Promega (Madison, WI). Lipofectamine 2000 transfection reagent was from Invitrogen (Calsbad, CA). All primers were obtained from IDT (Coralville, IA). Other cell culture reagents were purchased from Invitrogen or Sigma–Aldrich.

2.2. Plasmid constructs and site-directed mutagenesis

A series of pGL3-basic vectors containing the proximal ABCG2 promoter spanning −1285/+362, −628/+362, −312/+362, −243/+362, or −115/+362 in the multi-cloning site were generated as described previously [10]. A 3.8 kb PCR product spanning −3783/+977 was cloned into the NheI/HindIII sites of the pGL3-basic vector, termed BCRP-3.8 kb. Utilizing "GCGTG" as the core of AhR binding site, computer-based search has resulted in five potential AhR binding sites in the first 3.8 kb of ABCG2 promoter (Fig. 3A). Site-directed mutagenesis was accomplished using QuikChange Site-directed mutagenesis kit (Stratagene/Agilent Technologies, La Jolla, CA) with the following primers: AhRE1 (forward-5′CCC-GGCAGTCGGGGCTTGAACTCACCCCCGCCCGC 3′, reverse-5′GCGGG-CGGGGGTGAGTTCAAGCCCCGACTGCCGGG 3′); AhRE4 (forward-5′GGCCTCCCAAAGTACTAGGATTACAGCTTGAAGCCACCGTGCCTGG-CC 3′, reverse-5′GGCCAGGCACGGTGGCTTCAAGCTGTAATCCTAGT-ACTTTGGGAGGCC 3′), and AhRE5 (forward-5′GCTAGATGACACGT-TAGTGGGTGCAGTTGGACAGCATGGCACATGTATAC 3′, reverse-5′G-TATACATGTGCCATGCTGTCCAACTGCACCCACTAACGTGTCATCTAGC 3′). The newly generated reporter construct and all mutants were confirmed by sequence analysis. The pRL-TK vector (Promega, Madison, WI) expressing renilla luciferase was used as transfection internal control.

2.3. Transfection assays in LS174T cells

LS174T cells (from American Type Culture Collection, Manassas, VA) were seeded in 24-well plates at a density of 1.8×10^5 cells per well in DMEM supplemented with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin. Cells were transfected with one of the ABCG2 promoter constructs (as depicted in Figs. 3 and 4) and the pRL-TK by use of Lipofectamine 2000 following the manufacturer's instruction. Twenty-four hours after transfection, cells were treated with vehicle control (0.1% DMSO, v/v), or 3MC at the concentration of 1 and 5 (μM) for another 24 h. Cell lysates were harvested in 1× Reporter Lysis Buffer and assayed using Promega Dual Luciferase Reporter assay kit (Promega). Transfection efficiency was normalized to renilla luciferase and data were presented as mean ± SD of fold activation over control from three independent experiments.

2.4. Real-time PCR analysis

LS174T cells were seeded in 12-well plates at a density of 2.5×10^5 cells per well. After growing to approximately 80% confluence, cells were treated with vehicle control (0.1% DMSO) or test compounds at the concentrations as indicated in Fig. 1 for 24 h. Total RNA was isolated from treated cells using the RNeasy Mini Kit (Qiagen, Valencia, CA) and reverse-transcribed using the High Capacity cDNA Archive kit (Applied Biosystems Inc; Foster, CA) following the manufacturers' instruction. SYBR green real-time PCR assays were performed in 96-well optical plates on an ABI 7000 Sequence Detection System (Applied Biosystems Inc; Foster City, CA) to measure GAPDH (forward: 5′ CCCATCACCATCTTC-CAGGAG 3′, and reverse: 5′ GTTGTCATGGATGACCTTGGC 3′) and ABCG2 (forward: 5′-GCGACCTGCCAATTTCAAATG-3′, and reverse: 5′-GACCCTGTTAATCCGTTCGTTT-3′). Data were represented as fold induction calculated by the equation $2^{-\Delta\Delta C_t}$, where $\Delta\Delta C_t$ is the relative change in threshold cycle between control and treated samples.

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