Research Article

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Gender matters: Estrogen receptor alpha (ERα) and histone deacetylase (HDAC) 1 and 2 control the gender-specific transcriptional regulation of human uridine diphosphate glucuronosyltransferases genes (*UGT1A*)

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Background & Aims: Gender influences incidence, progression, and therapy of hepatogastrointestinal diseases. The aim of this study was to elucidate the molecular mechanism of gender-specific UDP-glucuronosyltransferases *(UGT1A)* regulation, representing important hepatogastrointestinal detoxification enzymes for xenobiotics, drugs, and endobiotics.

Methods: *UGT1A*-gene activation was studied by reporter gene experiments and estrogen receptor alpha (ESR1/ERα) co-transfection using KYSE70- and HepG2 cells (male origin), and SW403 cells (female origin). Cell lines, and humanized transgenic *UGT1A (htgUGT1A)* mice (female/male) were treated with the ERα inhibitor tamoxifen. *UGT1A* mRNA expression was analyzed by TaqMan PCR, the recruitment of ERα, histone deacetylases (HDAC), and the aryl hydrocarbon receptor (AhR) by chromatin immunoprecipitation (ChIP), and ERα expression in gastrointestinal mouse tissues by Western blot and immunofluorescence.

Results: In KYSE70 cells (male), *UGT1A* gene expression was induced 5–10 fold, and inhibited in the presence of ERα by 55–77%. In SW403 (female) cells, absent inducibility was restored after tamoxifen treatment. In the jejunum and colon of *tgUGT1A* mice, *UGT1A* induction that was exclusively detected in male mice could be restored in female mice after tamoxifen pre-treatment. ChIP assays demonstrated the recruitment of ERα and HDACs to the xenobiotic response elements of *UGT1A* promoters during gene repression. Western blot showed higher ERα expression in the female jejunum and colon.

Conclusions: We show gender-specific transcriptional control of *UGT1A* genes in jejunum and colon, which is repressed by ERα and the recruitment of HDCAs to the *UGT1A* promoter sequence in females. A molecular mechanism controlling gender-specific drug metabolism and its therapeutic reversal is demonstrated.

Keywords: Gender; Drug metabolism; UDP-glucuronosyltransferase.
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Abbreviations: UGT, UDP-glucuronosyltransferase; ERα, estrogen receptor alpha; AhR, arylhydrocarbon receptor; Nrf2, nuclear factor erythroid related factor 2; XRE, xenobiotic response element; TCDD, 2,3,7,8-tetrachlordibenzo-p-dioxin; tBHQ, *tert*-butylhydroquinone; PCR, polymerase chain reaction; HDAC, histone deacetylase; CYP, cytochrome P450 enzyme; PPARA, peroxisome proliferator-activated receptor alpha; $HNF4_{\alpha}$, hepatocyte nuclear factor 4 alpha; CAR, constitutive androstane receptor; PXR, pregnane X receptor.

Introduction

Gender-specific medicine is an emerging field dealing with differences in health, disease, and therapeutic options between men and women. Current disease management and treatment algorithms usually fail to specify gender-specific aspects although many examples exist regarding incidence and progression of diseases, as well as for gender-dependent drug pharmacokinetics. Clinically relevant examples of gender differences include autoimmune hepatitis, which affects women more often than men [1], and alcoholic disease characterized by a male predominance although women more rapidly develop alcohol-induced liver disease after consuming lower doses [2,3], and thus carry a greater risk of developing alcoholic hepatitis, fibrosis, and liver cirrhosis [4]. Data from animal research suggest that an increased female risk for liver damage may be linked to the female hormone estrogen, which among other functions can increase the sensitivity of hepatic Kupffer cells to endotoxin [5]. In contrast, the progression of hepatic fibrosis in chronic hepatitis B and C appears to be slower in women [6,7]. Gender also influences hepatic drug metabolism evidenced by acetaminophen clearance rates, which are 22% higher in men than in women because of different rates of glucuronidation [8].

A possible molecular mechanism of these observations is likely to be related to the differential regulation of hepatic genes, specifically those regulated by sex hormones. Gender differences in the expression of cytochrome P450 (CYP) and phase II enzymes including rodent UDP-glucuronosyltransferases (UGT) have been reported [9,10]. In this context, UGTs play an essential role for the detoxification of drugs [11] as well as a broad array of potentially

cytotoxic or genotoxic compounds including human carcinogens [12,13]. In this capacity, UGTs contribute to antioxidative protection [14,15]. The transcriptional regulation of UGTs proceeds by pathways including the xenobiotic-activated aryl hydrocarbon receptor (AhR), hepatocyte nuclear factor 4 alpha (HNF4$_\alpha$), constitutive androstane receptor (CAR), and pregnane X receptor (PXR) [11]. For these receptors, a steroid-dependent responsiveness has been reported in rodent models [16]. In addition, an interaction between AhR, which is an important transcriptional *UGT1A* gene activating factor, and the estrogen receptor alpha (ERα) was described as a responsible mechanism for the suppression of *CYP1A1* transcription in different studies [17], possibly by a direct physical interaction of ERα and AhR [18–20].

Previous data from our laboratory demonstrated the induction of human *UGT1A* transcription by oral coffee exposure, which was controlled by AhR and nuclear factor erythroid 2-related factor 2 (Nrf2) [15]. In humanized transgenic *(htg)UGT1A* mice, oral coffee administration led to strong *UGT1A* activation in the liver and stomach of both male and female mice but was unexpectedly absent in the jejunum and colon of female mice.

Based on these data, we hypothesized that *UGT1A* repression by ERα may play a regulatory role for the observed absence of AhR-mediated inducibility in the jejunum and colon of female *htgUGT1A* mice. In this study, we present a molecular mechanism for the role ERα plays in gender-specific regulation of drug metabolizing *UGT1A* genes that would be of considerable interest to the differences potentially encountered in detoxification capacity, cellular protection, and individual drug therapy between men and women.

Materials and methods

Chemicals and coffee preparation

2,3,7,8-Tetrachlordibenzodioxin (TCDD) was obtained from Wellington Laboratories (Berlin, Germany). *Tertiary* butylhydroquinone (*t*BHQ), 17β-estradiol (E_2), tamoxifen, and trichostatin A (TSA) were purchased from Sigma-Aldrich (Taufkirchen, Germany). Coffee was prepared as described before [15].

Cell culture conditions

Hepatoma (HepG2), colon carcinoma (SW403), and esophagus carcinoma (KYSE70) cells were grown in RPMI 1640 (HepG2 and KYSE70), or Dulbecco's modified Eagle's medium (SW403) supplemented with 10% fetal bovine serum. The cells were maintained at 37 °C under an atmosphere of 5% CO_2- 95% air.

Humanized transgenic UGT1A mice and treatment

The generation of the *htgUGT1A-WT* mouse line was described before [21]. Transgenic *UGT1A* mice were kept at the Central Animal Facility, Hannover Medical School, and bred to non-transgenic C57BL/6 mice (Charles River, Sulzfeld, Germany). All experiments were approved by the Local Institutional Animal Care and Research Advisory committee and authorized by the local state government of Lower Saxony, Germany. Male and female *htgUGT1A*-WT mice were treated with undiluted coffee as their drinking water for 3 days. Each control pool contained mice given normal drinking water. Female *htgUGT1A*-WT mice were pretreated with 50 mg/kg tamoxifen (i.p.) for 3 days followed by parallel treatment with 50 mg/kg tamoxifen (i.p.) and coffee for further 3 days.

RNA isolation and RT-PCR

Either 100 mg of cells or 100 mg of frozen tissue was homogenized in TRIzol (Invitrogen, Karlsruhe, Germany). Five μg of RNA was used for generation of cDNA in an oligo(dT)-primed Superscript III reverse transcriptase reaction according to the manufacturer's instructions (Invitrogen, Karlsruhe, Germany).

Quantitative real-time PCR

For gene expression analysis, cDNA concentrations were determined by qPCR relative to mouse beta-actin. TaqMan-PCR with gene specific primers and probes was performed in an ABI Prism 7000 Sequence Detection System (Applied Biosystems, Darmstadt, Germany) using qPCR MasterMix (Eurogentec, Cologne, Germany). Primer and probe sequences are listed in Supplementary Table 1. For quantification of *ESR1* gene expression TaqMan® Gene Expression Assay was used (Hs00174860_m1, Applied Biosystems, Darmstadt, Germany).

Luciferase assays

Luciferase assays were performed as previously described [14]. The generation of luciferase reporter gene constructs was described elsewhere [14,15,22]. For co-transfection, 300 ng (if not stated otherwise) of VP16-ER alpha plasmid (plasmid 11351, Addgene, Cambridge, USA) was co-transfected. The control plasmid VP16 was generated by excising the ESR1 insert using EcoRI restriction enzyme. Twenty-four h after transfection, cells were treated with coffee (12% in medium), E_2 (10 nM), tamoxifen (10 μM), TCDD (5 nM), tBHQ (100 μM) or solvent (EtOH, DMSO) and incubated for additional 48 h. Statistical analysis was performed using Student's *t*-test for comparisons between groups. Differences were considered significant when p values were below 0.05.

Western blot

One hundred mg of frozen mouse tissue was homogenized in extraction buffer (150 mM NaCL, 50 mM Tris-HCl, pH 7,4) and incubated on ice for 30 min. After centrifugation (15,000g, 10 min, 4 °C), the supernatant was used for Western blotting performed as previously described [15]. As primary antibody, anti-ERα (MA1-310, Thermo Scientific, Germany) was used.

Chromatin immunoprecipitation (ChIP)

Chromatin immunoprecipitation (ChIP) was performed using the ChIP Assay Kit from USB (Stauffen, Germany) according to the manufacturer's instructions. Chromatin samples derived from nuclear extracts of KYSE70 and SW403 cells were sonicated for 12 cycles, 30 s each. Immunoprecipitation was performed with 2 μg of anti-ERα (PAB12620, Abnova, Heidelberg, Germany), anti-AhR (H00000196-M02, Abnova), anti-HDAC1 (ab7028, Abcam), and anti-HDAC2 (ab12169, Abcam). An anti-UGT1A1 antibody (Santa Cruz Biotechnology, Inc.) was used as negative control. *UGT1A* promoter sequences were amplified by PCR using specific primers for an upstream and downstream portion of the 5′ upstream region of the respective gene (primer sequences are listed in Supplementary Table 2).

Immunofluorescence

Paraffin sections were deparaffinized with xylene, rehydrated, and incubated in PBS for 2 h. Slides were blocked in a 10% goat serum in PBST for 3 h at room temperature. The primary antibody (anti-ERα, ab30656, Abcam, Cambridge, UK) was used at a concentration of 1:50 in 5% serum-PBST at 4 °C overnight. The secondary antibody (Alexa Fluor® 488 Goat Anti-Rabbit IgG, Invitrogen, Karlsruhe, Germany) was used at 1:250 at room temperature for 1 h.

Results

ERα expression in HepG2, KYSE70, and SW403 cells depends on cell line gender

HepG2 cells were originally derived from liver tissue of a 15-year old Caucasian American male [23]. KYSE70 cells were established from a squamous cell carcinoma resected from middle intra-thoracic oesophagus of a 77-year old Japanese man [24]. By contrast, SW403-cells were derived from an adenocarcinoma of the bowel of a 51-year old Caucasian female [25]. The analysis of *ERα* mRNA in these cell lines showed that there is high ERα expression in the female-derived SW403 cell line and nearly no ERα expression in the HepG2 and KYSE70 cells of male origin (Fig. 1A).