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Novel identification of UDP-glucuronosyltransferase 1A10 as an estrogen-regulated target gene

Athena Starlard-Davenport[a], *Beverly Lyn-Cook*[b], *Anna Radominska-Pandya*[a,*]

[a] *Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, 4301 W. Markham, Slot 516, Little Rock, AR 72205, USA*

[b] *Division of Personalized Nutrition and Medicine, National Center for Toxicological Research, HFT-100, Jefferson, AR 72079, USA*

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ABSTRACT

Recently, we have shown that UGT1A10 is actively involved in the inactivation of E_1, E_2, and their 2- and 4-hydroxylated derivatives. In the present study, we show for the first time that treatment of the MCF-7 ER-positive breast cancer cell line with E_2 produces a dose-dependent up-regulation of UGT1A10 mRNA levels, followed by a steady down-regulation. In contrast, E_2 did not stimulate mRNA expression in the MDA-MB-231 (ER)-negative breast cancer cell line. Expression of UGT1A10 mRNA was blocked by the antiestrogen, ICI 182,780, but not by the transcriptional inhibitor, actinomycin-D. These findings suggest that regulation of UGT1A10 mRNA might be a primary transcriptional response mediated through the ER. Expression of UGT1A10 mRNA was also stimulated by other estrogenic compounds including propylpyrazoletriol (PPT) and genistein (Gen). Exposure of MCF-7 cells to 0.1 nM E_2 up-regulated, and then down-regulated, UGT1A protein and enzymatic activity toward E_2 at 10 nM E_2 as determined by Western blot and glucuronidation activity assays. Collectively, these results suggest that induction of UGT1A10 mRNA expression by E_2 might be mediated through ER, and that this isoform is a novel, estrogen-regulated target gene in MCF-7, ER-positive human breast cancer cells. The finding of E_2-induced expression of UGT1A10 mRNA, followed by the down-regulation of UGT1A10 at pharmacological concentrations of E_2, might have a significant moderating effect on E_2 availability for ER and estrogen clearance, thereby promoting the signaling of E_2 in breast cancer cells.

1. Introduction

Estrogen exposure is essential for the function and regulation of the female reproductive system, and for the development of female secondary sex characteristics. In the breast, estrogens stimulate the growth and differentiation of the ductal epithelium, induce mitotic activity of ductal cylindric cells, and stimulate the growth of connective tissue [1]. Moreover, estrogens can stimulate the growth of breast cancer cells [1,2]. The two most potent endogenous estrogens, estrone (E_1) and 17β-estradiol (E_2), are both ligands for estrogen receptors (ER). These receptors have a higher affinity for E_2 than for E_1, and E_2 is believed to be the predominant endogenous activator of ER-mediated cellular processes [3,4]. ER-mediated cellular events occur in estrogen-target tissues when an excess of free extra-cellular E_2 or other estrogenic ligands diffuse across the cell membrane and bind an ER. ER undergoes a conformational change to displace the heat shock protein (hsp) which encourages dimerization and tight binding of ER to its specific DNA target, the estrogen response element

* *Corresponding author*. Tel.: +1 501 686 5414; fax: +1 501 603 1146.
E-mail address: radominskaanna@uams.edu (A. Radominska-Pandya).
Abbreviations: UGT, UDP-glucuronosyltransferase.
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(ERE) [5]. The activated estrogen–ER complex can interact with cofactors that modify ER action either by enhancing (coactivators) or inhibiting (corepressors) target gene transcription [6]. This process results in the production of mRNAs that are translated to proteins that regulate many cellular processes [7,8]. In general, the regulation of estrogens in the breast is dependent upon many factors including the action of E_2 via ER and the activity of steroidogenic enzymes involved in the production and metabolism of estrogens.

Glucuronidation, catalyzed by UDP-glucuronosyltransferases (UGTs), is an important process of metabolism and detoxification of estrogens. UGTs are membrane-bound glycoproteins localized in the endoplasmic reticulum and nuclear envelope [9]. UGTs are defined by their ability to catalyze the transfer of glucuronic acid from the co-substrate UDP-glucuronic acid (UDP-GA) to a wide range of hydrophobic endogenous and exogenous substrates generating more polar, generally inactive molecules that can be readily excreted from the body in the urine or bile [10,11]. However, UGTs can also generate bioactive, perhaps toxic compounds, including those of steroid hormones, morphine, retinoids, and bile acids [12–16].

UGTs have been classified into two families, UGT1 and UGT2, based on the similarity of their primary amino acid sequences [17]. The UGT1 isoforms in humans are encoded by a single gene locus consisting of several first exons that are independently spliced to four common exons (2–5), resulting in enzymes with unique N-terminal domains and identical C-terminal domains [18]. The UGT1A isoforms display broad substrate specificity and are expressed predominantly in the liver, but are also found in extrahepatic tissues [17,19]. In contrast to the UGT1 family, the individual UGT2 genes each comprise six exons that are not shared between the UGT2 family members [20]. The UGT2B isoforms conjugate many compounds including bile acids, steroid hormones, and xenobiotics [21]. Because several UGT1A and UGT2B isoforms conjugate substrates that bind nuclear receptors, it has been suggested that these enzymes play a role in controlling their steady state concentrations for nuclear receptors and thereby maintain homeostasis of the cell [22].

Recently, UGT1A10 has been identified as the major UGT that conjugates estrogens and/ or their hydroxylated metabolites [23,24]. Human UGT1A10 is highly expressed in several tissues of the gastrointestinal tract (biliary tract, colon, stomach, and duodenum) [25]. As yet, unpublished data from our laboratory have shown that UGT1A10 is also expressed and functional in human breast tissues. UGT1A10 is highly homologous to UGT1A7 and UGT1A8 and all belong to the UGT1A7-10 cluster of *UGT1A* genes that share sequence similarities of approximately 90% [26]. UGT1A10 is known to conjugate estrogens, bioflavonoids, phenols, and polycyclic aromatic hydrocarbons (PAHs), which are carcinogenic [25]. The identification of UGT1A10 in conjugating estrogens and its expression in human breast tissues and cells is a novel finding.

Recently, UGT2B15, a UGT involved in the glucuronidation of steroids and xenobiotics was identified as a novel, estrogen-regulated gene in ER-positive human breast cancer cell lines, including MCF-7. Therefore, we hypothesized that UGT1A10, the major estrogen-conjugating UGT, might also be regulated by estrogens. In the present study, we investigated the effects of E_2 on UGT1A10 mRNA expression in the human breast adenocarcinoma cell lines, MCF-7, which express ERα/β, and MDA-MB-231, which lacks ER expression. We demonstrated that UGT1A10 mRNA expression is stimulated, and then decreased, by E_2 in a dose- and time-dependent manner. Furthermore, we postulate that this dose- and time-dependent regulation of UGT1A10 by E_2 might be mediated via the ER. This study shows for the first time, that UGT1A10 is an estrogen-target gene. The finding of E_2-induced expression of UGT1A10 mRNA might have an effect on E_2 availability for ER, thereby regulating E_2 signaling in estrogen-responsive breast cancer. The down-regulation of UGT1A10 at pharmacological concentrations of E_2 might have a significant moderating effect on estrogen clearance, thereby promoting the signaling of E_2 in breast cancer.

2. Materials and methods

2.1. Chemicals

[^{14}C]UDP-glucuronic acid was purchased from Perkin-Elmer Life and Analytical Sciences (Boston, MA). Estradiol (E_2), genistein (Gen), actinomycin-D, cycloheximide (CHX), propylpyrazoletriol (PPT), and saccharolactone (saccharic acid-1,4-lactone) were obtained from Sigma-Aldrich (St. Louis, MO). The antiestrogen, ICI 182,780, was supplied by ICI Pharmaceuticals (Macclesfield, England, Lot #C42710). All other chemicals and solvents were of the highest quality commercially available.

2.2. Cell culture

All cell lines used in these studies were obtained from the American Type Culture Collection (Manassas, VA). The human breast cancer cell line that expresses both ERα/β, MCF-7 (ATCC No. HTB-22), and the human ER-negative breast cancer cell line MDA-MB-231 (ATCC No. HTB-26), were routinely maintained in MEM medium supplemented with 5% FBS. Six days before harvesting, cells to be treated were washed with phosphate buffered saline (PBS) and incubated with phenol red-free MEM containing 5% charcoal dextran (CD)-treated FBS to strip the medium of any estrogens. Cells were treated with estrogens (E_2, PPT and Gen), antiestrogen (ICI 182,780), and the translational inhibitor, CHX, at concentrations given in the legends for Figs. 2–5. All treatment groups were harvested at the same time, and total RNA was prepared using the Qiagen RNA Isolation kit (Valencia, CA) according to the manufacturer's instructions. In transcription inhibitition assays, actinomycin-D (1 μg/ml) was added to the culture medium together with vehicle, E_2, PPT, or Genistein, and mRNA was collected at various time points, as shown in Fig. 6. All experiments were performed at least three times.

2.3. cDNA synthesis and quantitative real-time PCR (QRT-PCR)

cDNA was synthesized from total RNA using the Clontech cDNA kit (Clontech, Mountain View, CA). Briefly, total RNA (2 μg) from cells was reverse transcribed in a final vol-