Glucocorticoids Antagonize Estrogens by Glucocorticoid Receptor–Mediated Activation of Estrogen Sulfotransferase

Haibiao Gong,1 Michael J. Jarzyna,2 Timothy J. Cole,4 Jung Hoon Lee,1 Taira Wada,1 Bin Zhang,1 Jie Gao,6 Wen-Chao Song,5 Donald B. DeFranco,2 Shi-Yuan Cheng,6 and Wen Xie1,2

1Center for Pharmacogenetics and Department of Pharmaceutical Sciences, 2University of Pittsburgh Cancer Institute and Department of Pathology; 3Department of Pharmacology, University of Pittsburgh, Pittsburgh, Pennsylvania; 4Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria, Australia; and 5Institute for Translational Medicine and Therapeutics and Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania

Abstract

Glucocorticoids and estrogens are two classes of steroid hormones that have essential but distinct physiologic functions. Estrogens also represent a risk factor for breast cancer. It has been suggested that glucocorticoids can attenuate estrogen responses, but the mechanism by which glucocorticoids inhibit estrogenic activity is unknown. In this study, we show that activation of glucocorticoid receptor (GR) by dexamethasone (DEX) induced the expression and activity of estrogen sulfotransferase (SULT1E1 or EST), an enzyme important for the metabolic deactivation of estrogens, because sulfonated estrogens fail to activate the estrogen receptor. Treatment with DEX lowered circulating estrogens, compromised uterine estrogen responses, and inhibited estrogen-dependent breast cancer growth in vitro and in a xenograft model. We further showed that the mouse and human SULT1E1 genes are transcriptional targets of GR and deletion of Sult1e1/Est in mice abolished the DEX effect on estrogen responses. These findings have revealed a novel nuclear receptor–mediated and metabolism-based mechanism of estrogen deprivation, which may have implications in therapeutic development for breast cancers. Because glucocorticoids and estrogens are widely prescribed drugs, our results also urge caution in avoiding glucocorticoid–estrogen interactions in patients. [Cancer Res 2008;68(18):7386–93]

Introduction

Glucocorticoids and estrogens are two classes of steroid hormones essential to mammals. Glucocorticoids have been implicated in a variety of cellular processes, ranging from development to metabolism, immune response, and apoptosis. Endogenous glucocorticoids, such as cortisol in humans and corticosterone in rodents, are synthesized in the adrenal cortex under the control of the hypothalamic-pituitary-adrenal axis (1, 2). In addition to their physiologic functions, glucocorticoids are among the most commonly prescribed drugs for their antiinflammatory and immunosuppressive effect (1, 3). Glucocorticoids exert most of their functions by binding to the glucocorticoid receptor (GR; NR3C1), a member of the nuclear hormone receptor superfamily. In the absence of a ligand, GR resides in the cytoplasm associating with the heat shock protein hsp90 and several other proteins. Upon ligand binding, GR is dissociated with hsp90 and translocated into the nucleus, where it activates gene expression by binding to glucocorticoid-responsive elements (GRE) located in the promoter regions of target genes (4–6).

Estrogens are sex hormones essential for mammals’ reproduction. Although produced primarily in the ovary, estrogens circulate systemically and exert their effects on various reproductive and nonreproductive tissues (7, 8). The estrogen receptor (ER), also a nuclear hormone receptor, plays an important role in mediating estrogenic effects. The uterus is one of the organs highly responsive to estrogens. In mice, uterine estrogen responses include water imbibition and increased uterine weight, as well as transactivation of estrogen-responsive genes, such as c-fos and lactoferrin (Lff; ref. 9). In addition to their physiologic functions, estrogens also play an important role in the initiation and progression of human breast cancers (10–12). Accordingly, strategies to down-regulate estrogen activities have been effective to treat and prevent breast cancers (13, 14).

An important metabolic deactivation pathway for estrogens is the sulfotransferase (SULT)–mediated sulfation, because sulfonated estrogens cannot bind to and activate ER, thus losing their estrogenic activities (15). Sulfotransferases belong to a family of phase II drug metabolizing enzymes. Although several SULT isoforms have been shown to sulfonate estrogens in vitro, the estrogen sulfotransferase (SULT1E1 or EST) is believed to be the major SULT isoform responsible for estrogen sulfation at physiologic concentrations due to its high affinity at nanomolar concentrations of estrogens (16, 17). The importance of SULT1E1 in estrogen homeostasis and reproductive function was manifested in the Sult1e1/Est null mice (18). SULT1E1 expression has also been implicated in human breast cancer. SULT1E1 is highly expressed in normal human mammary epithelial cells, but its expression is diminished in breast cancer cells, including the ER-positive and estrogen-responsive MCF-7 cells (19). The differential expression of SULT1E1 in normal human mammary epithelial cells and breast cancer cells suggests that down-regulation of SULT1E1 may have led to unchecked estrogen stimulation and cancerous transformation of the breast epithelium. An ectopic expression of SULT1E1 in MCF-7 cells suppressed the estrogen response (20, 21), suggesting that reactivation of endogenous SULT1E1 gene expression may represent a novel therapeutic strategy to inhibit estrogen-dependent growth of breast cancer cells. It has been recognized that glucocorticoids inhibited estrogen responses (22–24). Treatment with dexamethasone (DEX), a synthetic glucocorticoid, attenuated the estrogen-induced uterine expression of insulin-like growth factor-I (IGF-I; ref. 25). DEX also
blocked the stimulatory effect of estrogen on MCF-7 cell proliferation (26). However, the mechanism by which glucocorticoids inhibit estrogenic activity is unknown.

In this study, we show that activation of GR by DEX induced the expression and activity of SULT1E1, which in turn facilitated estrogen deactivation and inhibited estrogen-dependent breast cancer growth in cell culture and in vivo. Our results have revealed a unique GR-mediated and metabolism-based hormone-hormone interaction. Moreover, the GR-mediated SULT1E1 gene activation may represent a novel strategy for estrogen deprivation that can be used to treat and prevent breast cancers.

Materials and Methods

Mouse models. The GR null (27), Sult1e1/Est null (28), and PXR null (29) mice have been described previously. The wild-type C57BL/6J mice were purchased from JAX Mice and Services. The ovarioctomized nude mice used in the xenograft model were purchased from Taconic. The use of mice in this study has complied with all relevant federal guidelines and institutional policies.

Uterotropic bioassay. Three-week-old virgin female mice were subjected to daily s.c. injections of vehicle (5% ethanol in PBS) or E2 (0.25 μg/kg) for 3 d, as previously described. Mice were then sacrificed 24 h after the last E2 dose, and the uteri were dissected and weighed. In the DEX-treated group, mice received daily i.p. injection of DEX (20 mg/kg) starting 3 d before the E2 treatment. Injections were continued until the completion of the experiments.

Uterine estrogen responses. Five-week-old mice were ovarioctomized and recovered for 1 wk before treatment with DMSO or DEX (20 mg/kg in DMSO) for 3 d. Mice were then given a single s.c. injection of E2 (0.25 μg/kg) and sacrificed 2 h after. Mice also received an i.p. injection of 5-bromo-2-deoxyuridine (BrdUrd; 60 mg/kg) 2 h before sacrifice. For each mouse, one uterine horn was processed for RNA extraction and real-time PCR analysis, and the other for paraffin section and immunostaining using an anti-BrdUrd antibody, as we have previously described (30).

Northern blot analysis and real-time reverse transcription–PCR. Total RNA was extracted using the TRIZOL Reagent from the Invitrogen. Northern hybridization was carried out as described (31). Total RNA was treated with RNase-free DNase I and reverse transcribed into single-stranded cDNA. Real-time PCR using the SYBR Green–based assay was performed with the ABI 7300 Real-Time PCR System (30). Estrogen sulfotransferase activity assay. Liver cytosols were prepared by homogenizing tissues in 5 mmol/L KPi buffer (pH 6.5) containing 0.25 mol/L sucrose. The cytosols were then used for sulfotransferase assay, which was carried out using [35S]phosphoadenosine phosphosulfate (PAPS; Perkin-Elmer) as the sulfate donor (30). In brief, 20 μg/mL of total liver cytosolic extract was incubated with 1 μmol/L of estrone substrate. After the reaction, free [35S]PAPS was removed by extracting with ethyl acetate. A 300-μL aliquot of the aqueous phase was then counted in the Beckman LS6500 scintillation counter for radioactivity.

Measurement of serum bioactive E2 level. The serum concentrations of active E2 were measured using the Ultra-Sensitive Estradiol RIA kit (DSL-4800) from Diagnostic Systems Laboratories, Inc., as previously described (30).

Cell transient transfection and luciferase activity assay. HepG2 cells were transfected with the reporter constructs and hGRα expression vector in 48-well plates, as previously described (30). When necessary, cells were treated with DEX (1 μmol/L) in media containing 10% charcoal-stripped serum for 24 h before the luciferase assay. The transfection efficiency was normalized against the β-gal activities from the cotransfected CMX-β-gal vector.

Human and mouse primary hepatocyte preparation and treatment. Human livers were obtained through the Liver Tissue Procurement and Distribution System (LTPADS), and hepatocytes were isolated by three-step collagenase perfusion (32). Mouse hepatocytes were also prepared by collagenase perfusion (33). Cells were plated on gelatin-coated T25 flask and maintained in the hepatocyte maintenance medium from Cambrex BioScience supplemented with 107 mol/L insulin, 50 μg/mL gentamicin, and 50 ng/mL amphotericin and incubated overnight to allow the cells to attach. Cells were then treated with appropriate drugs for 24 h before cell harvesting.

 Xenograft model. 1 × 105 of MCF-7/vascular endothelial growth factor (VEGF) cells were inoculated into the mammary fat pads of 7-wk-old ovarioctomized female nude mice that were implanted with E2 pellets (0.72 mg/60-day release) or placebo pellets from the Innovative Research of America. The E2–treated mice were randomly divided into three groups, with two groups receiving a daily treatment of DEX at different doses (2 or 6 mg/kg) and the other group receiving vehicle. The volumes of the tumors were measured using a caliper every 5 d. Mice were labeled with BrdUrd for 30 min before sacrifice. Serum, tumor, and liver tissues were collected for analysis.

Electrophoretic mobility shift assay and chromatin immunoprecipitation analysis. Electrophoretic mobility shift assay (EMSA) was performed using receptor proteins prepared with the TNT in vitro transcription and translation system and [32P]-labeled double-stranded DNA fragments, as described previously (30). DMSO-treated or DEX-treated primary hepatocytes were used for chromatin immunoprecipitation (ChIP) assays using a GR antibody (sc9992) from Santa Cruz, as we described previously (30). The PCR primers for mouse Sult1e1/GRE are 5′-GCTCTGTTTGTGTAAGCAGTC-3′ and 5′-CCAAAATCCAGCGCTT-CAAAGG-3′. The primers for human SULT1E1/GRE are 5′-TAACCTTGTTCCACACAAA-3′ and 5′-TTTGGTGGAACACAAGTTTA-3′. The primers for Sult1e1/liver X receptor (LXR) response element (LXRE) are 5′-CCAAAGGGAAGACAGCTG-3′ and 5′-GAGAAGGAGGGAGACTAC-3′. The primers for Cyp7b1/RORE response element (RORE) are 5′-AACCTTAGAGGAGGAGCCATGAA-3′ and 5′-TGGATAATCCTCATGTGCAATGAGA-3′ (34). The PCR products of Sult1e1/GRE, SULT1E1/GRE, Sult1e1/LXRE, and Cyp7b1/RORE were 252, 174, 142, and 125 bp, respectively.

Statistical analysis. When applicable, results are presented as means ± SD. Animal numbers are labeled or shown in figure legends. The Student’s t test was used to calculate P values. P < 0.05 was considered statistically significant.

Results

Treatment with DEX induced the hepatic expression of Sult1e1 in a GR-dependent manner and decreased the circulating level of estrogens. In our effort to identify nuclear receptors and their ligands that control the expression of Sult1e1 in mice, we found that treatment with DEX in wild-type mice induced the mRNA expression of Sult1e1 in the liver (Fig. 1A). The basal expression of Sult1e1 mRNA in the liver is low. The hepatic Sult1e1 mRNA induction in DEX-treated mice was so dramatic that its expression reached a level similar to that of the testis (Fig. 1A), the tissue known to have the highest constitutive expression of Sult1e1 (15). DEX is known to activate both GR and the pregnane X receptor (PXR; ref. 35). The DEX effect on Sult1e1 mRNA induction was intact in PXR null mice (29) and the PXR agonist PCN failed to increase Sult1e1 mRNA level in wild-type mice (Fig. 1A). The lack of the PXR effect was also supported by the lack of Sult1e1 mRNA induction in transgenic mice that expressed a constitutively activated PXR in the liver (data not shown). Instead, by using GR null mice (27), we showed that the DEX effect on Sult1e1 mRNA expression was GR-dependent, because the Sult1e1 mRNA induction by DEX was completely abolished in GR null mice (Fig. 1B). Moreover, the DEX-induced Sult1e1 mRNA expression was suppressed by the coadministration of RU486, a known GR antagonist (Supplementary Fig. S1). The DEX effect on Sult1e1 mRNA expression seemed to be tissue specific. DEX had little effect...
on Sult1e1 mRNA expression in the testis, a tissue known to express GR (Fig. 1B; ref. 36). Consistent with Sult1e1 mRNA induction, increased estrone sulfation was observed in liver cytosols prepared from DEX-treated wild-type mice (Fig. 1C). Moreover, treatment of 4-wk-old intact virgin female mice with DEX for 3 days resulted in a significantly reduced circulating estradiol level (Fig. 1D).

DEX inhibited mouse uterine estrogen responses in a Sult1e1/Est-dependent manner. The uterus is highly responsive to estrogen stimulation. Therefore, we used uterine estrogen responses to examine the biological consequence of DEX-induced Sult1e1 expression. Treatment with 17β-estradiol (E₂) almost doubled uterine weight in intact wild-type mice (Fig. 2A). The uterotrophic effect of E₂ was largely abolished when the mice were simultaneously treated with DEX, whereas DEX alone had little effect (Fig. 2A). The uterine epithelial proliferation and gene expression were examined in ovariectomized virgin female mice by BrdUrd labeling and real-time PCR, respectively. As shown in Fig. 2B, treatment with E₂ caused a dramatic increase of BrdUrd labeling index (Fig. 2B, b), but this effect was abolished in mice pretreated with DEX (Fig. 2B, d). The inhibitory effect of DEX was abolished in Sult1e1/Est null mice (Fig. 2B, f), suggesting that Sult1e1 played an essential role in this inhibition. Similar effects of DEX and Sult1e1 null background were observed when uterine gene expression was evaluated. As shown in Fig. 2C, compared with vehicle control, treatment with E₂ increased the mRNA expression of progesterone receptor, Llf, and Igf-1, and suppressed Tnxip expression in wild-type mice, as expected (37–40). Pretreatment with DEX abolished E₂-responsive expression of all four genes in wild-type mice, but this inhibition was abrogated in Sult1e1/Est null mice.

DEX induced SULT1E1 mRNA expression in human hepatocytes and MCF-7 cells and inhibited E₂-stimulated MCF-7 cell proliferation. The effect of DEX on human SULT1E1 mRNA expression was investigated in primary human hepatocytes and breast carcinoma MCF-7 cells. DEX treatment up-regulated SULT1E1 mRNA expression in both cell types (Fig. 3A). Overexpression of GR in MCF-7 cells by transfection further enhanced DEX-induced SULT1E1 mRNA expression (Fig. 3A, right). MCF-7 cells are ER-positive and estrogen responsive. As expected, E₂ treatment induced ~2.5-fold increase in MCF-7 cell proliferation, as measured by BrdUrd labeling assay. Although DEX alone had little effect, it inhibited E₂-stimulated MCF-7 cell proliferation in a dose-dependent manner (Fig. 3B). The inhibition was specific for E₂-dependent breast cancer cell growth, because DEX had little effect on the proliferation of MDA-MB-231, an ER-negative and E₂-independent breast cancer cell line (Fig. 3B). The induction of SULT1E1 mRNA expression by DEX in MCF-7 cells was
GR-dependent. Knocking-down of GR by small interfering RNA (siRNA), as confirmed by real-time PCR (Fig. 3C) and Western blot analysis (data not shown), compromised DEX-induced SULT1E1 expression (Fig. 3C). DEX failed to inhibit E2-stimulated cell proliferation in GR siRNA-transfected MCF-7 cells (Fig. 3D).

DEX inhibited estrogen-dependent, but not estrogen-independent, human breast cancer cell tumorigenicity in nude mice. The induction of SULT1E1 and inhibition of estrogenic activity prompted us to determine whether DEX can inhibit estrogen-dependent breast cancer tumorigenicity in vivo by using the MCF-7 cell xenograft model. Due to the low tumorigenicity of the parent MCF-7 cells, we chose the MCF-7/VEGF cells that overexpress VEGF. The MCF-7/VEGF cells exhibited a higher penetrance of estrogen-independent growth and robust estrogen-dependent promotion (41). Ovariectomized mice were used to minimize the effect of endogenous E2. The DEX effect was evaluated in the presence of E2 supplied by implanted E2 pellets. Consistent with our previous report (41), the MCF-7/VEGF cells were highly tumorigenic in response to E2 stimulation (Fig. 4A). Compared with the vehicle treatment group, daily treatment with DEX (2 or 6 mg/kg) significantly decreased tumor volume. The difference was noticeable as early as 10 days after tumor cell inoculation, and the inhibition was sustained thereafter. The DEX effect was dose-dependent, with a more dramatic tumor inhibition observed when the higher dose (6 mg/kg) was used (Fig. 4A).

Shown in the insert of Fig. 4A are representative tumors from vehicle-treated and DEX-treated mice. The reduced tumor size in DEX-treated mice was associated with a decreased cell proliferation rate, as shown by BrdUrd labeling and immunostaining (Fig. 4B).

Consistent with the tumor inhibition phenotype, DEX treatment decreased the serum level of E2 in a dose-dependent manner (Fig. 4C), which was associated with an increased SULT1E1 mRNA expression in the liver and xenograft tumor tissues (Fig. 4D). In the same animals, the mRNA expression of several other hepatic estrogen metabolizing enzymes, including Cyp1a2, Cyp3a11, and Ugt1a1 (42), was not induced by DEX (Fig. 4D). Although high doses of DEX (20–50 mg/kg) can activate PXR in vivo (29), the DEX doses (2–6 mg/kg) we used in the xenograft model had little effect on PXR activation and, thus, failed to activate PXR target genes, such as Cyp3a11 and Ugt1a1 (43). These results suggested that both local (in the xenograft tumor) and systemic (in the liver) activation of SULT1E1 may have contributed to E2 deprivation and tumor inhibition.

SULT1E1 is a transcriptional target of GR. To understand the molecular mechanism by which GR regulates SULT1E1 gene expression, we have cloned and analyzed the mouse and human SULT1E1 gene promoters. Putative GREs were found in both the mouse and human promoters (Supplementary Fig. S2A). EMSA showed that GR can bind to both the mouse Sult1e1/GRE (Supplementary Fig. S2B) and human SULT1E1/GRE (Supplementary Fig. S2C). In both cases, the bindings could be efficiently competed by excess unlabeled wild-type Sult1e1 (SULT1E1)/GRE or MTV/GRE (a prototypical GRE), but not by Sult1e1 (SULT1E1)/GRE mutants (Supplementary Fig. S2B and C). ChIP assay using a GR-specific antibody showed that GR protein can be recruited to the GRE in the mouse Sult1e1 promoter, and DEX treatment enhanced the recruitment (Supplementary Fig. S2D). The recruitment of GR was GRE-specific, because no binding was detected

Figure 2. DEX inhibited mouse uterine estrogen responses in a Sult1e1/Est-dependent manner. A, quantitation of estrogen uterotrophic bioassay results. Three-week-old wild-type virgin female mice were treated with vehicle or DEX (20 mg/kg) for 3 d before being mock treated or treated with a single dose of E2 (5 μg/kg). Each group contained five mice. *, P < 0.05; **, P < 0.01, compared with the vehicle control. B, BrdUrd immunostaining on uterine paraffin sections from mice of different genotypes and drug treatments. All mice were ovariectomized 7 d before estrogen treatment. Mice received a single s.c. injection of vehicle or E2 (20 mg/kg) 2 h before being sacrificed. Mice were labeled for BrdUrd (60 mg/kg) for 2 h before sacrifice. Percentages of BrdUrd-positive nuclei are quantitated and labeled. When applicable, mice were pretreated with DEX for 3 d before E2 treatment. The original magnification is 200× for all panels. C, regulation of uterine gene expression as measured by real-time PCR. Pgr, progesterone receptor; Txinp, thioredoxin interacting protein. **, P < 0.01, compared with the wild-type vehicle control. Three mice were used for each group in B and C.
when PCR primers specific for LXRE on the Sult1e1 promoter (30) or RORE on the Cyp7b1 promoter (34) were used (Supplementary Fig. S2D). ChIP assay showed that GR was also recruited to the human SULT1E1/GRE encompassing promoter region, but not to a control promoter region that was distal from the SULT1E1/GRE site (Supplementary Fig. S2E). Consistent with EMSA and ChIP results, synthetic thymidine kinase (tk) reporter genes that contain 700-bp promoter sequences encompassing Sult1e1/GRE (Sult1e1, 700 bp) or three synthetic copies of Sult1e1/GRE (GRE*3), but not its mutant variant (Sult1e1/GRE-M*3), were activated by GR in the presence of DEX (Supplementary Fig. S2F). The GR-mediated Sult1e1/GRE*3 activation was comparable with that of a synthetic tk reporter gene containing three copies of the GRE derived from the rat tyrosine aminotransferase gene (Supplementary Fig. S2F), a known GR target gene. LXR had no effect on this GRE*3 reporter gene (data not shown). A synthetic tk reporter gene that contains three copies of the human SULT1E1/GRE, but not its mutant variant, was also activated by GR in a ligand-dependent manner (Supplementary Fig. S2G).

Discussion

It has been recognized that glucocorticoids inhibited the uterine responses stimulated by estrogens (22, 44). DEX treatment has also been shown to attenuate the estrogen-induced uterine Igf-1 and hepatic ER expression in ovariectomized rodents (25). However, the molecular basis for the antagonistic effect of glucocorticoids on estrogens has been elusive. Our results suggest that transcriptional activation of SULT1E1 by the DEX-GR pathway plays an important role in DEX-mediated estrogen deprivation. Our conclusions are supported by the observations that (a) DEX induces SULT1E1 gene expression in a GR-dependent manner (Fig. 1), (b) SULT1E1 is necessary for the estrogen deprivation effect of DEX (Fig. 2), (c) SULT1E1 is a transcriptional target of GR (Supplementary Fig. S2), and (d) a forced expression of SULT1E1 is sufficient to inhibit E2 activity in transient transfection and reporter gene assay (data not shown).

The current study has revealed an important role of SULT1E1 in glucocorticoid-induced estrogen deprivation. As the major SULT
isoform to sulfonate estrogens under physiologic conditions, SULT1E1 has been recognized for its role in estrogen deprivation. The critical role of SULT1E1 in estrogen homeostasis was shown by the disruption of the Sult1e1 gene in mice. Sult1e1/Est null males exhibited structural and functional lesions in their reproductive system, a phenotype resulting from chronic estrogen stimulation (28). In female Sult1e1/Est null mice, the defect in estrogen deprivation caused estrogen excess, leading to placental thrombosis and spontaneous fetal loss (18). In humans, ectopic expression of SULT1E1 in MCF-7 cells reduced the response to physiologic concentrations of estradiol and inhibited estrogen-stimulated DNA synthesis and cell proliferation (19, 20).

In spite of the extensive biochemical characterization and functional studies of SULT1E1 (18, 19), until recently, little has been known about the transcriptional regulation of this SULT isoform. We have recently shown that LXR, a nuclear receptor known for its function in lipid homeostasis and inflammation (45), activated Sult1e1/Est gene expression in the mouse liver (30). In the current study, we showed that the expression of SULT1E1 is also subjected to the transcriptional regulation by GR. The regulation of Sult1e1 by GR and LXR in the mouse liver seemed to be independent. The DEX and LXR agonists had an additive effect on activating hepatic Sult1e1 mRNA expression (data not shown). There are several notable differences between GR and LXR in their regulation of SULT1E1. First, the DEX effect on SULT1E1 mRNA expression can be seen in the liver and MCF-7 cells; whereas the LXR effect was limited to the liver (30). Second, the DEX effect can be seen in both mouse and human cells, including human hepatocytes and breast cancer cells; whereas the LXR effect seemed to be mouse-specific (30), reminiscent of the rodent-specific Cyp7a1 regulation by LXR (46). The DEX/GR-mediated SULT1E1 induction was also consistent with the observation that the hypercorticosteronemia in db/db C57BL/KsJ mice was associated with a marked increase in Sult1e1 mRNA expression in the liver (47). It has also been reported that DEX treatment in mice induced hepatic Sult1e1 gene expression (48, 49). However, neither the molecular mechanism nor the
physiologic implication of this regulation has been systematically evaluated in previous studies. The stimulatory role of estrogens in breast cancer initiation and progression has been well recognized. Accordingly, strategies to down-regulate estrogen activities have been proved effective to treat and prevent breast cancers (13, 14). These include the use of antiestrogens to prevent estrogen binding and use of aromatase inhibitors to inhibit estrogen synthesis. Activation of estrogen-metabolizing enzymes represents an independent strategy to down-regulate estrogenic activity. The latter notion was supported by the observation that SULT1E1 expression is decreased in many human breast cancer cell lines (19) and an ectopic expression of SULT1E1 in MCF-7 cells was sufficient to inhibit estrogen-dependent growth response. A more recent study showed that SULT1E1 levels were inversely correlated with the tumor size or lymph node status in breast cancer patients (50). Our results clearly showed that pharmacologic activation of GR was sufficient to restore SULT1E1 expression and inhibit MCF-7 cell growth in cell culture and in vivo. We propose that GR and SULT1E1 may represent novel therapeutic targets to down-regulate estrogen activity. Indeed, the inhibition of estrogen-dependent MCF-7/VEGF tumor growth by a GR agonist supports the practicality of this therapeutic strategy.

DEX is known to trigger insulin resistance and diabetes after a prolonged (2–5 months) drug treatment (51). The longest DEX treatment in our xenograft model was 35 days; the phenotype was obvious by day 10 of treatment (Fig. 4). In short-term experiments, we found that treatment with DEX (4 mg/kg) for 4 days modestly, but significantly, lowered serum glucose levels (data not shown). Therefore, we do not feel that the in vivo DEX treatments used in our study produced diabetic conditions. The aryl sulfotransferase/SULT1A1 can also sulfonate estrogens. The rat liver SULT1A1 gene expression has been shown to be glucocorticoid-inducible (52, 53). Interestingly, the DEX effect on SULT1A1 expression seemed to be species specific. Alnouti and Klaasen showed that DEX (75 mg/kg) had little effect on the expression of SULT1A1 in mice (49). The expression of human SULT1A1 was also not affected by DEX (54). In our experiments, treatment of female wild-type C57BL/6J mice with DEX (4 mg/kg) for 4 days decreased the mRNA expression of Sult1a1, whereas treatment of nude mice with DEX (6 mg/kg) for 5 weeks had little effect on the mRNA expression of Sult1a1 (Supplementary Fig. S3). These results suggest that induction of Sult1a1 may not play a role in our mouse models.

Other than their implications in breast development and breast cancer, estrogens are known to have diverse roles in many other tissues, including bone, liver, the central nervous system, and the vascular system (7, 8). Most, if not all, hormone replacement therapy regimens contain estrogens (55). Meanwhile, glucocorticoids are among the most frequently prescribed antiinflammatory and immunosuppressive drugs. The inhibition of estrogenic activity by glucocorticoids raises the concern of hormone-hormone interactions in clinical practice. For example, it may be desirable to limit or be cautious about using glucocorticoids in patients who have undergone hormone replacement therapies. In conclusion, GR-mediated estrogen deprivation may have broad implications in estrogen homeostasis, endocrine therapies, and tumorigensese, including a potential use in breast cancer prevention and treatment.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Acknowledgments

Received 4/24/2008; revised 7/5/2008; accepted 7/10/2008.

Grant support: NIH grants ES014626 and CA107011 (W. Xie) and Department of Defense Breast Cancer Program grants DAMD17-01-1-0975 and DAMD-17-02-1-0584 (S.Y. Cheng). H. Gong is supported by a Postdoctoral Fellowship (PDF5030488) from the Susan G. Komen Breast Cancer Foundation.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Normal human hepatocytes were obtained through the Liver Tissue Procurement and Distribution System, Pittsburgh, Pennsylvania, which was funded by NIH contract N01-DK-7-0004/HHSN267200700004C.

We thank Yongong Zhai, Shaheen "Sear" Khadem, and Diana Xie for technical assistance, and Dr. Stephen Strom (University of Pittsburgh Department of Pathology) for providing human hepatocytes.

References
24. Rhen T, Grissom S, Afshari C, Cidlowska JA. Dexamethasone blocks the rapid biological effects of...


Glucocorticoids Antagonize Estrogens by Glucocorticoid Receptor–Mediated Activation of Estrogen Sulfotransferase

Haibiao Gong, Michael J. Jarzynka, Timothy J. Cole, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/68/18/7386

Supplementary Material
Access the most recent supplemental material at:
http://cancerres.aacrjournals.org/content/suppl/2008/09/11/68.18.7386.DC1

Cited articles
This article cites 55 articles, 18 of which you can access for free at:
http://cancerres.aacrjournals.org/content/68/18/7386.full#ref-list-1

Citing articles
This article has been cited by 18 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/68/18/7386.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.