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

Haibiao Gong, Michael J. Jarzynka, Timothy J. Cole, Jung Hoon Lee, Taira Wada, Bin Zhang, Jie Gao, Wen-Chao Song, Donald B. DeFranco, Shi-Yuan Cheng, and Wen Xie

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):7386–7393]
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 ovariectomized 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.

Uterotrophic bioassay. Three-week-old virgin female mice were subjected to daily s.c. injections of vehicle (5% ethanol in PBS) or E2 (5 μg/kg/d) 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 s.c. 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 ovariectomized 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 (20 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 and a half uteri were used for RNA extraction and real-time PCR analysis, 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 (sc8992) from Santa Cruz, as we described previously (30). The PCR primers for mouse Sult1e1/GR are 5'-GGTCCCTGTGTTGTAGCAGCTC-3' and 5'-CCACAACTCCGCGCTTCCAAGG-3'. The primers for human SULT1E1/GR are 5'-TAACTTGTGTCACACAAA-3' and 5'-TTTTGTGTTGAGCACAAGTITA-3'. The primers for Sult1e1/liver X receptor (LXR) response element (LXRE) are 5'-CACAAGGGGAACAGGTGCT-3' and 5'-GAGAAGGGACGACAGACTAC-3'. The primers for Cyp7b1/ROE response element (ROE) are 5'-AACCTATAGAGAGGACCCATGAA-3' and 5'-TGATGAATCTACA-3'. The PCR products of Sult1e1/GR, SULT1E1/GR, Sult1e1/LXRE, and Cyp7b1/ROE 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 progesterone X receptor (PXR; ref. 35). The DEX effect on Sult1e1 mRNA expression was PXR-independent, because the 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 Tnnp 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. 2A, 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).

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.

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 can 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...
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

Figure 4. DEX inhibited estrogen-dependent human breast cancer cell tumorigenicity in nude mice. A, growth kinetics of the MCF-7/VEGF tumors in the presence of indicated hormone and drug treatments. The DEX treatment groups received daily treatment of DEX at indicated doses by i.p. injection. Tumor volumes were measured at the indicated times. Results are presented as means ± SD. Each group contains at least nine mice. *, P < 0.05; **, P < 0.01, compared with the DEX treatment group. Shown in the insert are representative E2-induced tumors in vehicle-treated or DEX-treated mice with the DEX doses labeled. B, BrdUrd immunostaining in E2-induced tumors 25 d postinoculation. Quantitation of BrdUrd-positive nuclei is labeled. Three mice of each group from A were selected for BrdUrd immunostaining. C, serum levels of E2 from mice in A at the time of sacrifice (35 d postinoculation). *, P < 0.05. At least five mice of each group were used for E2 measurement. D, DEX treatment increased hepatic and MCF-7/VEGF tumor expression of Sult1e1/SULT1E1, but not for Cyp1a2, Cyp3a11, and Ugt1a1. **, P < 0.01. Three mice of each group were used for gene expression analysis.
physiological 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 Klaassen 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 tumorigenesis, including a potential use in breast cancer prevention and treatment.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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