The relevance of estrogen receptor-β expression to the antiproliferative effects observed with histone deacetylase inhibitors and phytoestrogens in prostate cancer treatment

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Abstract

In the prostate, estrogen receptor β (ERβ), the preferred receptor for phytoestrogens, has features of a tumor suppressor. To investigate the mechanisms underlying the beneficial effects on prostate cancer of histone deacetylase inhibitor valproic acid (VPA) and phytoestrogen tectorigenin, we analyzed the expression of ERβ after tectorigenin or VPA treatment. For further functional analysis, we knocked down ERβ expression by RNA interference. LNCaP prostate cancer cells were treated with 5 mmol/L VPA or 100 μmol/L tectorigenin and transfected with small interfering RNA (siRNA) against ERβ. Control transfections were done with luciferase (LUC) siRNA. Expression of ERβ was assessed by Western blot. mRNA expression was quantitated by real-time reverse transcription-PCR. Expression of ERβ mRNA and protein markedly increased after VPA or tectorigenin treatment. When ERβ was knocked down by siRNA, the expression of prostate-derived Ets factor, prostate-specific antigen, prostate cancer–specific indicator gene DD3^{PCA3}, insulin-like growth factor-1 receptor, the catalytic subunit of the telomerase, and ERα was up-regulated and the tectorigenin effects were abrogated. ERβ levels were diminished in prostate cancer and loss of ERβ was associated with proliferation. Here, we show that siRNA-mediated knockdown of ERβ increases the expression of genes highly relevant to tumor cell proliferation. In addition, we show that one prominent result of treatment with VPA or tectorigenin is the up-regulation of ERβ resulting in antiproliferative effects. Thus, these drugs, by restoring the regulatory function of ERβ in tumor cells, could become useful in the intervention of prostate cancer. [Mol Cancer Ther 2007;6(10):2626–33]

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Introduction

In Western societies, prostate cancer is the most common malignancy in men, whereas, obviously due to dietary influences, Asian men have much lower incidences of prostate cancer than their Western counterparts (1). This protection against prostate cancer in Asian societies is attributed to weak dietary estrogens such as isoflavonoids, flavonoids, and lignans contained in vegetarian food (e.g., soy products). These estrogens of plant origin, termed phytoestrogens, have a considerable affinity to estrogen receptors and bind generally in favor of the β isoform of estrogen receptors (2). The expression status of the estrogen receptors at the mRNA and protein levels has been investigated intensely, sometimes with conflicting results (3–5). Nevertheless, it is widely accepted that estrogen receptor β (ERβ) adopts a regulatory role in estrogen signaling, mediating antiproliferative effects. ERβ restrains the transcriptional and proliferative activation conducted by ERα (6) and also modulates androgen receptor signaling (7, 8). ERα is predominantly expressed in the stroma of the prostate and thus mediates effects via paracrine pathways. ERβ is the estrogen receptor in prostate epithelium and shows decreased expression in prostate cancer. Many authors agree that ERβ exerts a protective effect against aberrant cell proliferation and carcinogenesis (9–12). Each of the prostate cancer cell lines LNCaP, PC-3, and DU-145, according to Lau et al. (13), expresses ERβ, whereas only PC-3 cells express ERα. However, Linja et al. (5) found low mRNA expression of ERα in all three cell lines and reported detectable levels of ERβ. Similar features for LNCaP cells were found by Ito et al. (3). Loss of ERβ expression at both the transcriptional and translational levels occurs during prostatic carcinogenesis and tumor progression, albeit metastatic prostate cancer cells may regain ERβ expression (4, 9, 10, 14). In a recent population-based prospective study on Japanese men, Kurahashi et al. (15) observed a dose-dependent decrease in the risk of localized prostate cancer with consumption of soy isoflavones. However, they also found that the effects of isoflavones differed by stage of prostate carcinoma, which became evident in an increased risk for advanced prostate cancer associated with one of the soy products investigated. As one explanation for this surprising effect, the authors suggest the protective role of ERβ, which is partially or completely lost in cancers

with higher metastatic potential (15). The antiproliferative, anti-invasive, and proapoptotic properties of ERβ indeed have been associated with the function of a tumor suppressor (16) and, therefore, the restoration of ERβ, which is lost during carcinogenesis due to gene silencing, is a desirable goal for prostate cancer therapy. Gene silencing and consequent aberrantly repressed gene expression in cancers due to epigenetic events (i.e., DNA methylation and histone deacetylation) can be reversed by several drugs, which might represent therapy approaches for prostate cancer (17, 18). Recently, we introduced the therapeutic potential of valproic acid (VPA) for prostate cancer, which featured inhibition of histone deacetylase activity in LNCaP prostate cells (19). VPA is an established anticonvulsant that has been characterized as an inhibitor of histone deacetylases (20). As a consequence of VPA-induced histone deacetylase inhibition, we found increased expression of insulin-like growth factor (IGF)–binding protein 3 and tissue inhibitor of matrix metalloproteinases-3 in LNCaP prostate cancer cells. In another study, we showed that phytoestrogens such as the isoflavone tectorigenin can also rectify the abnormal expression of key elements responsible for the malignancy of prostate cancer (21). Tectorigenin binds to both estrogen receptors, ERα and ERβ, albeit with a higher affinity for ERβ, and has selective estrogen receptor modulator activities (22). Here, we report that a common denominator in the beneficial effects from these drugs is the increase of ERβ expression. Recently, the molecular mechanisms underlying ERβ actions in androgen-independent DU-145 (23) and androgen-responsive LNCaP (24) prostate cancer cells have been investigated using small interfering RNA (siRNA). To further elucidate ERβ functions and phytoestrogen effects, we conducted knockdown analyses by siRNA-mediated silencing of ERβ expression. This study revealed the capacities of the phytoestrogen tectorigenin as well as of the histone deacetylase inhibitor VPA to reestablish ERβ expression. siRNA-mediated silencing of ERβ reversed the effects of both compounds and abrogated the beneficial effects elicited by tectorigenin.

Materials and Methods

Cell Culture

Human prostate cancer cell lines PC-3 and LNCaP between passages 30 and 40 were grown in phenol red–free RPMI 1640 (PAN-Systems GmbH) containing 10% FCS (PAA), 1% L-glutamine, 2% amino acid solution, and 1% penicillin-streptomycin. For VPA and TG stimulation, cells were suspended in 5-mL medium at ~60% confluence using 50-mL culture flasks and allowed to seed overnight. To exclude para-estrogen effects, for VPA and TG stimulation, cells were grown in DMEM. For siRNA transfection, LNCaP cells were grown in RPMI medium. Tectorigenin (Girindus) was dissolved in DMSO as a 1,000× stock solution and added at a final concentration of 10, 50, and 100 μmol/L, respectively, to DMEM. An equivalent volume of DMSO was added to control flasks. Cells were incubated for 24 h at 37°C and 5% CO_2 in a humidified incubator. Na-valproat (Sigma) was dissolved in DMEM at a final concentration of 1, 5, or 10 mmol/L, respectively. After 24 h incubation, cells were harvested for RNA and protein extraction or used for cell viability assay. Treatment of LNCaP cells with the antiestrogen ICI 182,780 (100 nmol/L) was carried out for 24 h with ethanol as solvent.

siRNA Transfection

Cells were plated in six-well plates 24 h before transfection with two different siRNA oligonucleotides against ERβ (Stealth siRNA duplex oligoribonucleotides, Invitrogen). In control transfections, we used LUC siRNA against the luciferase gene (Eurogentec). We used Oligofectamine reagent (Invitrogen) and Opti-MEM (Invitrogen) according to the manufacturers' recommendations. The day before transfection, cells were seeded in six-well plates at a density of 8×10^4 per well with RPMI 1640, washed with PBS (BioWhittaker) immediately before transfection, and delivered in 800 μL fresh RPMI medium. After adding the transfection components, 5 μL of Oligofectamine reagent, 185 μL of Opti-MEM, and 10 μL of a 20 μmol/L stock solution of oligonucleotides per well, cells were incubated for 48 h, and then diluted with 800-μL RPMI and incubated for another 20 h. Cells were harvested 68 h after initiation of transfection. Two different siRNA oligo sets were used: ERβ siRNA 1, 5′-CCAUCGCCAGUUAUCACAUCUGUAU-3′ and 5′-AUACAGAUGUGAUAACUGGCGAUGG-3′; ERβ siRNA 2, 5′-GCGAUU ACGCAUCGGGAUATT-3′ and 5′-UAUCCCGAUGCGUAAUCGCTT-3′.

Western Blots

Protein expression was assessed by Western blot analysis with 3 μg/mL mouse anti-ERβ (Genetex), mouse anti-ERα (Santa Cruz Biotechnology, Inc.), and mouse anti–α-tubulin (Sigma-Aldrich) monoclonal antibodies. After 68 h of incubation, transfected LNCaP cells were homogenized with lysis buffer containing 150 mmol/L NaCl, 1 mmol/L EDTA, 50 mmol/L Tris-HCl (pH 7.4), 1% NP40, 0.25% sodium deoxycholate, 1 mmol/L phenylmethylsulfonyl fluoride, 1 μg/mL aprotinin, 1 μg/mL leupeptin, 1 μg/mL pepstatin A, 1 mmol/L Na_3VO_4, and 1 mmol/L NaF. After centrifugation, cell lysates were boiled and denatured in sample buffer containing SDS and DTT (Invitrogen). NuPAGE 4% to 12% Bis-Tris precast gel and MES buffer (Invitrogen) were used for electrophoresis. After electrotransfer onto polyvinylidene difluoride membrane (GE Healthcare), protein-bound membrane was hybridized with the above-mentioned antibodies. For visualization, we used horseradish peroxidase–coupled secondary antibodies (Dianova) and the ECL Plus kit (GE Healthcare).

RNA Extraction and cDNA Synthesis

Cells were detached with trypsin and washed with PBS, and then total cellular RNA was extracted with RNeasy Mini Kit (Qiagen) from pelleted LNCaP cells. The integrity and quantity of isolated RNA was measured by Agilent Bioanalyzer 2100 with a RNA 6000 Nano LabChip-Kit (Agilent Technologies). Isolated, total cellular RNA was reverse transcribed with Omniscript RT Kit (Qiagen).

Real-time Reverse Transcription-PCR

cDNA resulting from reverse transcription was used for mRNA quantification by reverse transcription-PCR (iCycler, Bio-Rad). For all primer sets, a 20-μL PCR reaction, using a SYBR Green RT-PCR Kit (Eurogentec), was subjected to 2 min 50°C, 10 min 95°C, then 40 cycles of 15 s 95°C and 1 min for individual primers at annealing temperature, followed by a melting curve analysis to prove specificity of the PCR. In all experiments, housekeeping gene acidic ribosomal protein (*ARP*) served as an internal control. Quantitative PCR was done as previously published (19). Primers for ERβ were the Quantitect Primer Assay Hs_ESR2_1_SG (Qiagen). ERα sequence was taken from ref. 25 and prostate-derived Ets factor (PDEF), prostate-specific antigen (PSA), prostate cancer–specific indicator gene $DD3^{PCA3}$ (DD3), porphobilinogen deaminase (PBGD), and the catalytic subunit of the telomerase (hTERT) were used as previously described (21). Primers for the housekeeping gene *ARP* were forward primer, 5′-CGACCTGGAAGTCCAACTAC-3′, and reverse primer, 5′-ATCTGCTGCATCTGCTTG-3′.

Cell Viability, Proliferation, and Cytotoxicity Assays

For VPA and TG treatments (24 h; 5,000 cells per well), a colorimetric bromodeoxyuridine test (Roche Diagnostics GmbH) was done according to the manufacturer's instructions. For siRNA transfections, cells were transfected as described and incubated for 62 h in 96-well plates; cell viability was measured with an Alamar Blue assay (Biosource).

Statistical Analyses

Statistical calculations, EC_{50} values, mean ± SD, and *P* values were carried out with GraphPad Prism software version 2.0 and calculated using the unpaired nonparametric *t* test at 95% confidence interval and using Mann-Whitney *U* tests with $P < 0.05$ considered statistically significant.

Results

VPA as well as Tectorigenin Treatment Causes an Increase of ERβ Expression

In an attempt to identify the initiation of the antiproliferative and proapoptotic effects accompanied by changes in gene expression observed with VPA and tectorigenin treatments, we quantitated ERβ expression in prostate cancer cells. As shown in Fig. 1A, VPA or tectorigenin evoked marked increases of ERβ mRNA expression in LNCaP prostate cancer cells. In contrast, the housekeeping gene *ARP* did not respond to such treatments (Fig. 1B). In concordance with elevated ERβ mRNA expression, protein expression was also significantly increased, whereas the expression of the housekeeping gene *α-tubulin* remained constant (Fig. 1C). VPA or tectorigenin treatment had an opposite effect on ERα expression. As shown in Fig. 1D, ERα expression was down-regulated in the same experiments where ERβ had been up-regulated by these drugs.

Treatment of LNCaP cells with various concentrations of VPA also caused a marked decrease of cell proliferation with a maximum effect at 5 mmol/L, whereas tectorigenin decreased cell proliferation significantly at concentrations >50 μmol/L (Fig. 1E). A combination of 1 mmol/L VPA pretreatment for 24 h and then tectorigenin (10–100 μmol/L) for another 24 h did not reveal an additive effect on cell proliferation (data not shown).

Elimination of ERβ Expression in LNCaP Cells

To verify that ERβ expression is central to the effects observed with VPA and tectorigenin treatments, we next carried out functional analyses on this receptor molecule. First, we tried to eliminate ERβ function at the protein level using the antiestrogen ICI 182,780. Surprisingly, ICI 182,780 also caused diminished mRNA expression of ERβ and also caused reduced mRNA expression of PDEF, PSA, DD3, IGF-I receptor, hTERT, and ERα (Supplementary Table S1A).[4] In an attempt to selectively knock down ERβ expression without apparent side effects, we applied RNA interference with siRNA oligos targeted at ERβ expression. We used two different siRNA sequences against ERβ, which both eliminated ERβ expression to a similar extent in a range of 10% to 30% residual expression (data not shown). The following experiments were done with ERβ siRNA 2 oligos itemized in Material and Methods. As evident in Fig. 2A and B, transfection of LNCaP cells with ERβ siRNA 2 oligos caused a marked knockdown of ERβ expression at the mRNA and protein levels. The ERβ knockdown with siRNA had no immediate effects on tumor cell viability (Fig. 2C). In addition, off-target effects from siRNA 2 oligos could be excluded because ERα, with a sequence very homologous to ERβ, was not knocked down by these siRNA oligos (Fig. 3F and Fig. 3H).

Changes of Gene Expression Induced by VPA or Tectorigenin Are Reversed by ERβ Knockdown

The knockdown of ERβ expression with siRNA caused an altered expression status for several genes. These alterations deflect into the opposite direction as those following VPA or tectorigenin treatment. Hence, PDEF, PSA, DD3, IGF-I receptor, and hTERT, the expressions of which were shown to be down-regulated after VPA or tectorigenin treatment, were up-regulated by ERβ knockdown (Fig. 3A–E). Furthermore, ERβ knockdown induced the expression of ERα, which is normally repressed in LNCaP cells (Fig. 3F).

Thus, siRNA-mediated knockdown of ERβ caused an opposite effect compared with VPA or tectorigenin treatment where, concomitant with increased ERβ expression, the ERα expression was down-regulated (Fig. 1D). Housekeeping gene *ARP* (Fig. 2A) did not change its expression status following such treatments. The same was true for the low-expressed housekeeping gene *PBGD* (Fig. 1G).

ERβ Knockdown Abolished the Effects of Tectorigenin Treatment

Two prominent effects of tectorigenin treatment in LNCaP cells are concomitant PDEF and PSA decreases.

[4] Supplementary material for this article is available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).

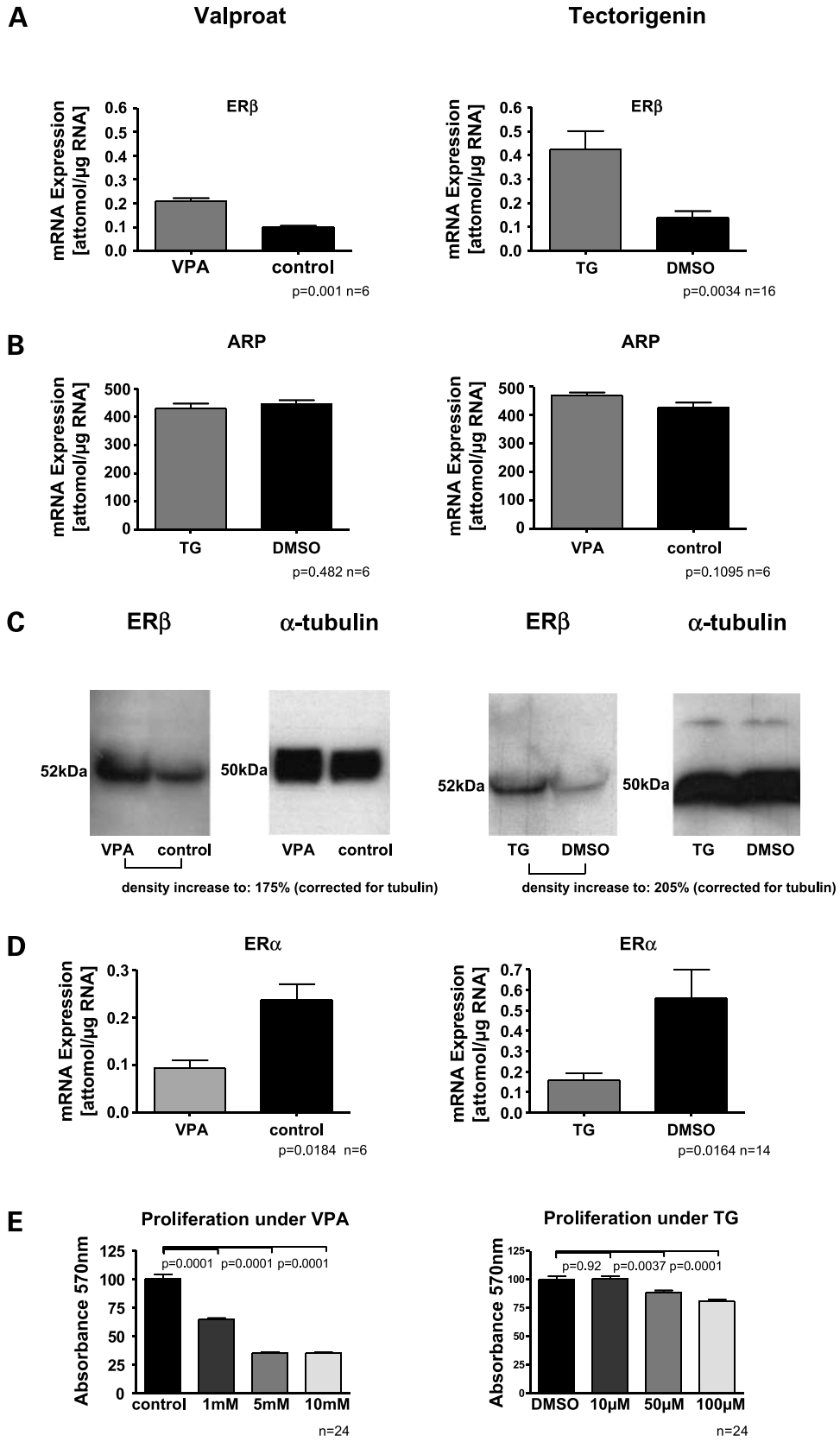

Figure 1. Effects of VPA or tectorigenin treatment of LNCaP cells on ERβ and ERα expression and cell proliferation. ERβ and ERα mRNA expression after treatment of LNCaP cells with VPA or tectorigenin, respectively, compared with control stimulations done with medium alone or DMSO, respectively (**A** and **D**). Effects of the same treatment as in (**A**) on housekeeping gene *ARP* (**B**). *Columns,* mean mRNA expression; *bars,* SD. *P* and *n* values are indicated. Western blot analyses of ERβ and α-tubulin protein expression after VPA or tectorigenin treatment versus control treatment as indicated, with calculated α-tubulin – corrected ERβ reduction (**C**). Bromodeoxyuridine proliferation test of LNCaP cells treated with varying concentrations of VPA or TG versus controls was done as described in Materials and Methods (**E**).

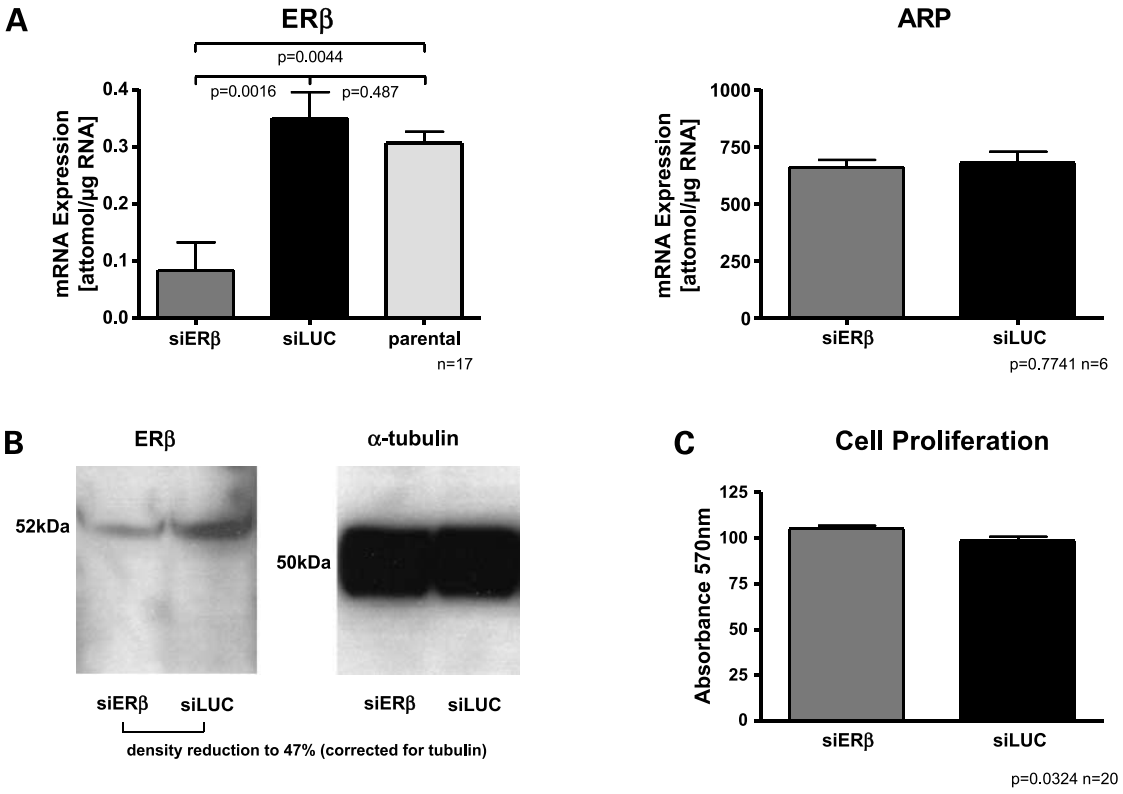

Figure 2. ERβ expression and cell proliferation after ERβ siRNA knockdown. **A,** mRNA expression of ERβ and ARP after knockdown of ERβ with siRNA in LNCaP compared with control transfections with LUC siRNA and untreated parental cells. *Columns,* mean mRNA expression; *bars,* SD. *P* and *n* values are indicated. **B,** Western blot analysis of ERβ protein expression after knockdown of ERβ with siRNA compared with control transfections with LUC siRNA, with calculated α-tubulin – corrected ERβ reduction. **C,** cell proliferation test of LNCaP cells treated with siERβ versus siLUC controls was done as described in Material and Methods.

These effects were markedly abolished when LNCaP cells were deprived of ERβ by a preceding siRNA-mediated knockdown of ERβ expression. As evident from Fig. 4, the usual decrease of PDEF and PSA caused by tectorigenin treatment was considerably impaired when ERβ had been knocked down before (*right columns*). However, in siLUC control–transfected cells, the tectorigenin effect was still detectable (*left columns*). Such an effect of an ERβ abolishment could not be shown for ICI pretreatment because the expression of PDEF and PSA was decreased by ICI treatment per se (Supplementary Table S1B).[4]

Discussion

In the present study, we introduced the potential of histone deacetylase inhibitors and phytoestrogens to up-regulate ERβ expression in prostate cancer cells and showed the consequences of a knockdown of this receptor. ERβ plays a pivotal role in carcinogenesis of the prostate and a decreased expression is associated with progression into pathologic stages of the disease. Restoring ERβ by adenoviral delivery in ER-negative DU-145 cells caused decreased invasiveness and growth and increased apoptosis of the prostate cancer cells, which reveals features of a tumor suppressor for ERβ (16). This became evident in the most recent study of Kurahashi et al. (15) with a seemingly contradictory conclusion. They found that intake of soy-derived isoflavones decreased the risk of localized prostate cancer but increased the risk of an advanced disease. As an explanation, it might suffice that in the latter, ERβ expression is lost and cannot convey the protection from phytoestrogens. Therefore, an intrinsic potential for an increase of ERβ expression might restore the therapeutic potential of phytoestrogens.

Epigenetic silencing, the abnormal repression of gene expression during cancer development, is generally caused by two distinct events: CpG island hypermethylation and histone hypoacetylation. In this study, we showed that the histone deacetylase inhibitor VPA caused reexpression of ERβ; moreover, the sole treatment of LNCaP cells with the phytoestrogen tectorigenin sufficed to restore ERβ expression. Although the ERβ promoter region shows a typical CpG island (18), we showed that inhibition of histone deacetylases alone yielded a considerable restoration of ERβ expression. This fact will ease the therapeutic application of ERβ up-regulation in prostate cancer, especially because VPA has a long-standing reputation as an anticonvulsant with manageable side effects. The use of VPA in rather high concentrations (e.g., 5 mmol/L) is common for *in vitro* studies to evoke immediate effects. In humans, VPA

is used in daily doses as high as 50 mg/kg (26). In previous studies, we showed that histone deacetylase activity is reduced significantly with VPA concentrations of 1 and 5 mmol/L and that trichostatin A, an established histone deacetylase inhibitor, caused similar effects in LNCaP cells (19). This study also revealed an induction of apoptosis due to treatment of LNCaP with VPA, indicated by a strong caspase-3 activity and DNA fragmentation. In terms of therapeutic applicability, the phytoestrogen tectorigenin is promising. Pure phytoestrogens are currently under investigation in clinical trials with safe doses of the phytoestrogen silibinin of 4 g/d (27). We used a phytoestrogen extract from *Belamcanda chinensis*, in which tectorigenin is a major component, in mice in doses of 1.5 g/kg of body weight without adverse effects for the test animals (21). In case of VPA, the up-regulation of ERβ expression most probably is due to a reversion of gene silencing by the histone deacetylase inhibitory properties of this drug.

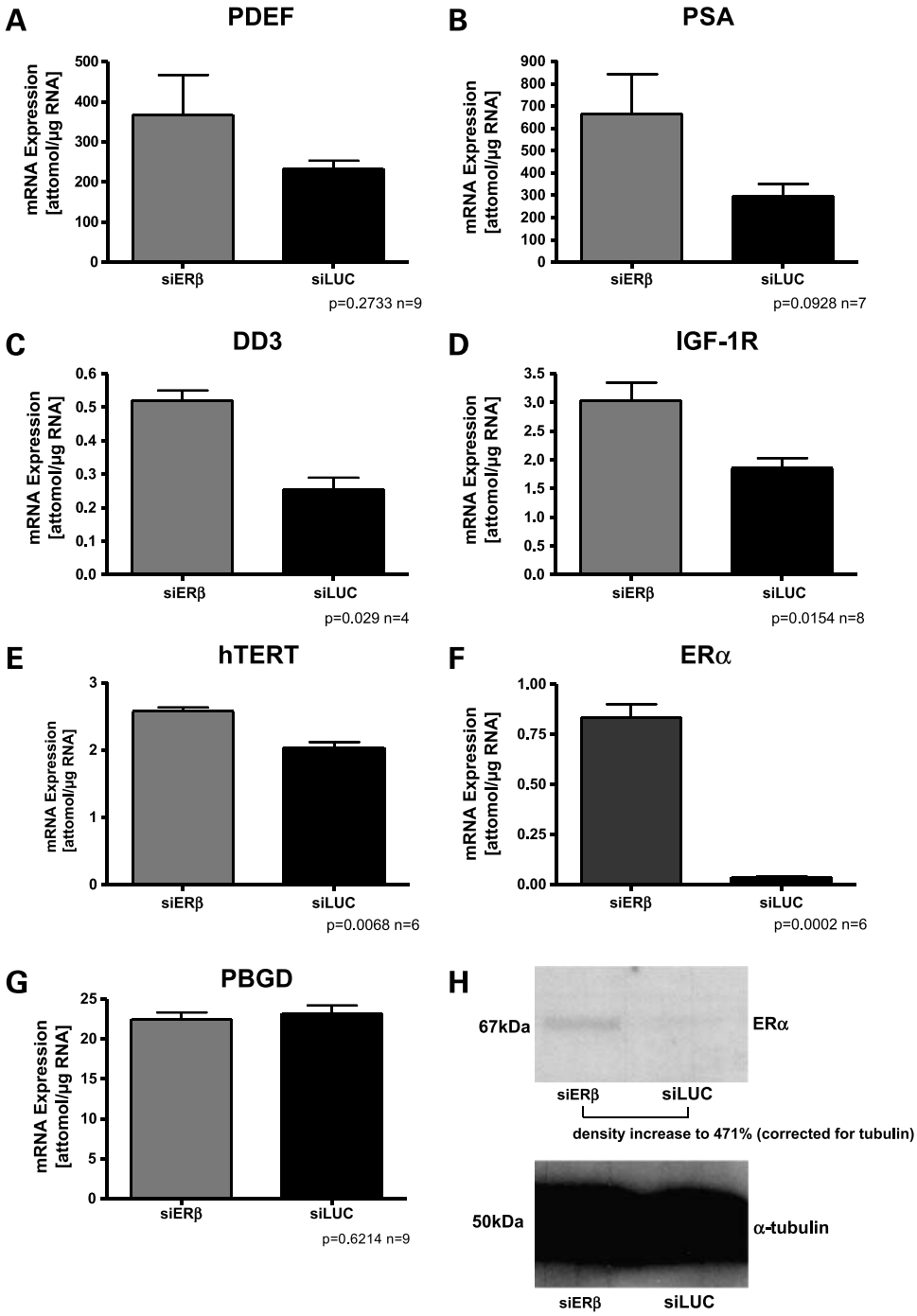

Figure 3. Altered gene expression resulting from ERβ knockdown. mRNA expression of PDEF (**A**), PSA (**B**), DD3 (**C**), IGF-1 receptor (**D**), hTERT (**E**), ERα (**F**), and PBGD (**G**) after knockdown of ERβ with siRNA in LNCaP compared with control transfections with LUC siRNA. *Columns,* mean mRNA expression; *bars,* SD. *P* and *n* values are indicated. **H,** Western blot analysis of ERα and α-tubulin protein expression after knockdown of ERβ with siRNA compared with control transfections with LUC siRNA, with calculated α-tubulin–corrected ERα increase.

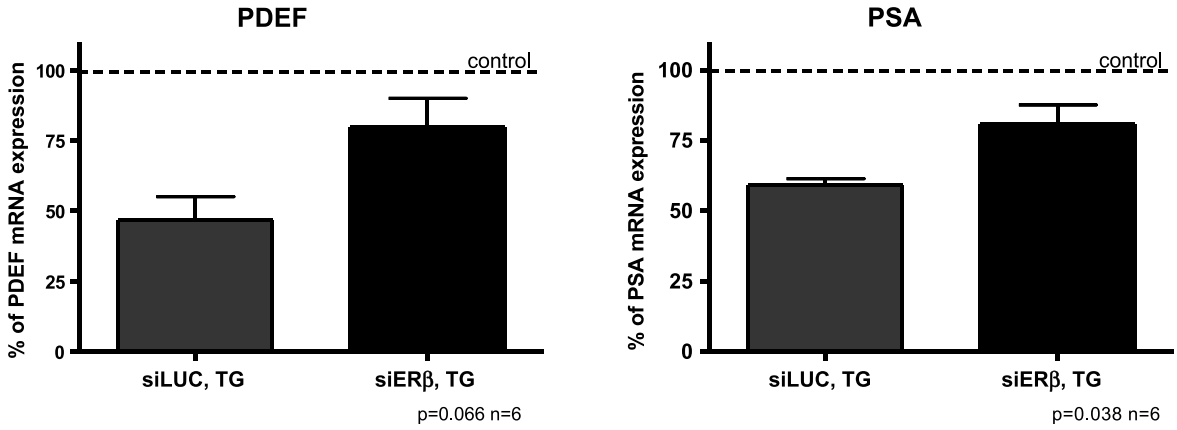

Figure 4. Abolishment of tectorigenin effects after ERβ knockdown. Percent mRNA expression of PDEF and PSA after siRNA knockdown of ERβ or LUC, respectively, in LNCaP cells, which were subsequently treated with 100 μmol/L tectorigenin. One hundred percent level (*dotted line*) represents control experiments with cells treated with siRNA for ERβ or LUC, respectively, and subsequently treated with solvent DMSO alone instead of tectorigenin. *Columns,* mean mRNA expression of three independent experiments; *bars,* SD. *P* and *n* values are indicated.

Because tectorigenin has selective estrogen receptor modulator activities, one explanation for the mechanism behind the up-regulation of ERβ seen here might be a feedback loop, which involves binding, modulation, and elevated transcription of this estrogen receptor. We have several lines of evidence that the phytoestrogen tectorigenin exerts its effects via ERβ and not via ERα or the mutant androgen receptor in LNCaP cells. We showed that tectorigenin has a strong affinity to both estrogen receptors with a preference for ERβ (22), and Bektic et al. (7) showed a very low affinity for isoflavones of the mutant androgen receptor. Furthermore, estradiol, activating the mutant androgen receptor in LNCaP, caused increased PSA expression, which is not seen for tectorigenin. In addition, PC-3 prostate cancer cells, which do not express an androgen receptor but the coactivator PDEF, showed a down-regulation of PDEF expression similar to LNCaP cells on tectorigenin treatment in the absence of an androgen receptor (data not shown).

Inhibition of histone deacetylase activity by VPA caused a decrease of tumor cell proliferation, decreased expression of PDEF and PSA, and increased expression of IGF-binding protein 3 and tissue inhibitor of metalloproteinase-3 in LNCaP cells (19). Such effects also occurred when LNCaP cells were treated with the phytoestrogen tectorigenin. In addition, tectorigenin diminished the expression of androgen receptor, DD3, IGF-1 receptor, and hTERT (21). In the present study, we found that a common denominator in both treatments is the up-regulation of ERβ expression. To further clarify the succession and molecular mechanism behind these events, we tried to elucidate the role of ERβ herein by blocking the remaining ERβ function of LNCaP cells. We failed to apply the antiestrogen ICI 182,780 in this capacity. Because of its marked antiproliferative and proapoptotic effects (13), ICI 182,780 obviously does not qualify for pretreatment studies and subsequent experiments in the same cell culture.

Pretreatment with RNA interference to eliminate ERβ has one advantage over pretreatment with a conventional drug to this end: experiment and control experiment share the same strain exposure caused by such pretreatments. The only difference between experiment and control is the sequence of the siRNA (i.e., siERβ or siLUC, respectively). Therefore, we applied RNAi to solely exclude ERβ expression and carried out functional analysis by means of siRNA-mediated silencing of ERβ. ERβ-specific RNA interference decreased expression of ERβ mRNA and protein without immediate alteration of cell viability. Silencing of ERβ by siRNA indeed impaired the effect of tectorigenin treatment as indicated by the expression of PDEF and PSA. siRNA silencing of ERβ without tectorigenin induced ERα expression and markedly increased the expression of PDEF, PSA, DD3, IGF-1 receptor, and hTERT. Thus, decreased ERβ expression is accompanied by the increase of key elements in carcinogenesis, whereas ERβ restoration caused a decrease of these functions. In addition, ERβ down-regulation abolished the beneficial effects seen with tectorigenin treatment. The relevance of these factors to prostate cancer, which obviously respond to the level of ERβ, is well characterized in the literature. PDEF as a coactivator of the androgen receptor that regulates the activity of the PSA promoter is a strong indicator for cancer cell malignancy and shows a better tumor association than Her2/neu, CA-125, Bcl-2, survivin, or telomerase (28, 29).

DD3 is a prostate-specific gene that is overexpressed in more than 95% of prostate cancer. This gene is used as a marker for prostate cancer and is considered for interventions targeting exclusively cells of malignant transformation (30). Deregulated vascular endothelial growth factor, hTERT, or components of the IGF-axis have a well-defined effect on many cancers including prostate cancer. hTERT activity is known to be regulated by estrogen receptor signaling (31). Finally, the IGF-1 receptor plays a pivotal role in ligand-independent androgen receptor activation in hormone-refractory disease, is overexpressed in prostate cancer, and persists in the metastatic disease

(32), and ERα has proproliferative features that are restrained by ERβ (6).

In conclusion, we showed that drugs with very different chemistries, such as short fatty acid acting as a histone deacetylase inhibitor as well as phytoestrogen isoflavones, cause an up-regulation of ERβ expression and therefore transform prostate cancer cells into a less malignant phenotype. Our application of RNA interference added more insights into the role of ERβ in phytoestrogen treatment of prostate cancer cells and further characterized the function of ERβ in prostate cancer. Future examinations of histone deacetylase inhibitors in combination with phytoestrogens are warranted.

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