Modulating Estrogen Receptor-related Receptor-α Activity Inhibits Cell Proliferation

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High expression of the estrogen receptor-related receptor (ERR)-α in human tumors is correlated to a poor prognosis, suggesting an involvement of the receptor in cell proliferation. In this study, we show that a synthetic compound (XCT790) that modulates the activity of ERRα reduces the proliferation of various cell lines and blocks the G1/S transition of the cell cycle in an ERRα-dependent manner. XCT790 induces, in a p53-independent manner, the expression of the cell cycle inhibitor p21wafl/cip1 at the protein, mRNA, and promoter level, leading to an accumulation of hypophosphorylated Rb. Finally, XCT790 reduces cell tumorigenicity in Nude mice.

Breast cancer is the most common malignancy in women (1). In the initial phases, these tumors often depend on estrogens for their growth. At the molecular level, 17β-estradiol (E2, ‡) the main estrogen in the adult) acts by up-regulating the expression of cyclin D1, which regulates the activity of cyclin-dependent kinases (cdks) 4 and 6 (2, 3). These cdks facilitate the transition from the G1 to the S phase of the cell cycle by hyperphosphorylating Rb (4). Adjuvant therapies aimed at counteracting the effects of estrogens are widely used (5). Anti-estrogens such as tamoxifen down-regulate the expression of cyclin D1 (3), whereas ICI182,780 (fulvestrant and faslodex) stimulates the tamoxifen down-regulate the expression of cyclin D1 (3), whereas ICI182,780 (fulvestrant and faslodex) stimulates the expression of p21wafl/cip1 (hereafter referred to as p21) (6). This protein blocks the activity of the cyclin D-cdk complexes leading to hypophosphorylation of Rb and arrest in the G1 phase of the cell cycle (4). However, a large proportion of breast cancers evolve so as to become resistant to anti-hormonal treatments through mechanisms that are not clearly characterized (1, 5). This illustrates the need of new targets against which to develop innovative anti-cancer therapies.

The effects of estrogens are mediated by two specific nuclear receptors (ERα and ERβ), which act as ligand-dependent transcription factors (7). Estrogen receptor-related receptors (ERRα, -β, and -γ) are other members of the nuclear receptor superfamily that share a high level of sequence identity with the ERs (8). However, no natural ligand has been identified for the ERRs, which are therefore considered as orphan receptors. Crystallographic studies have shown that ERRα and -γ spontaneously adopt active conformations (9, 10). Although ERRα regulates transcription in a constitutive manner, some of its activities (DNA binding and contact with coactivators) can be regulated by phosphorylation (11). Furthermore, compounds such as the phytoestrogen genistein can inhibit the transcriptional activities of ERRα (12). In addition, based on its capacity to disrupt the interactions between ERRα and the PGC-1α coactivator, the synthetic molecule XCT790 has been identified as an ERRα-specific inverse agonist (13). This compound down-regulates the expression of ERRα target genes probably by inducing an actively repressing conforma-

In this study we show that modulating the activity of ERRα using the specific compound XCT790 inhibits the proliferation of hormone-dependent and -independent cell lines as well as tumorigenesis in Nude mice. In cell culture, XCT790 blocks transition from the G1 to the S phase of the cell cycle. This is accompanied by an elevation in the expression of p21 protein and mRNA. The activity of the p21 promoter is stimulated by ERRα, and in this context, XCT790 acts as a super-agonist.

EXPERIMENTAL PROCEDURES

Cells and Hormonal Treatments—The human mammary epithelial cell lines MCF7 (WT and derivatives) and MDA-MB-231 and the human prostatic carcinoma PC-3 cell line were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum. Before experiments involving treatment with estrogens, MCF7 cells were cultured in hormone-free medium (phenol red-free Dulbecco’s modified Eagle’s medium) with 10% charcoal-stripped fetal bovine serum for 3
days and switched to medium containing 3% charcoal-stripped fetal bovine serum upon hormone treatment. The human prostatic carcinoma cell line LNCaP was cultured in RPMI 1640 medium supplemented with 10% fetal calf serum. For transduction of MCF7 cells, lentiviral particles expressing short hairpin RNA for p53 (pRS-p53; see Ref. 30) were produced by transient transfection of 293T cells using a calcium phosphate transfection technique. For transduction, 10^5 MCF7 cells were incubated for 24 h in complete medium. Prior to infection, medium was removed, and cells were incubated with viral supernatant for 24 h at 37 °C in the presence of 8 μg/ml protamine sulfate. After 5 days, transduced cells were selected by addition of 400 μg/ml hygromycin (Invitrogen) in the culture medium. In all experiments, XCT790 (3-[4-(2,4-bis-trifluoromethylbenzyl-oxy)-3-methoxyphenyl]-2-cyano-N-(5-trifluoromethyl-1,34]-thiadiazol-2-yl)-acrylamide was used at the concentration of 5 μM except for dose-response experiment. XCT790, E2, and epidermal growth factor were purchased from Sigma.

**Proliferation and Cell Cycle**—Cell proliferation was evaluated using MTS kit (Promega, Charbonniere, France) or cell counts and BrdUrd enzyme-linked immunosorbent assay (Roche Diagnostics) following the manufacturer’s instructions. Cells were plated in 96-well plates at a density of 10^4 cells per well. For cell cycle analysis, cells were resuspended in phosphate-buffered saline containing 25 μg/ml propidium iodide and 10 μg/ml RNase A (Sigma) after overnight fixation in 70% ethanol. Analysis was performed using a FACScan (BD Biosciences) and the CellQuest software (BD Biosciences).

**Expression Analysis**—siRNAs directed against ERRα (Dharmacon, Brebières, France; and Invitrogen) were transfected into MCF7 using Oligofectamine (Invitrogen) 72 h before hormonal treatments. Control siRNAs were from Dharmacon and Invitrogen (medium GC Stealth RNA interference negative control duplexes). RNAs were isolated by guanidinium thiocyanate/phenol/chloroform extraction. Total RNA was converted to first strand cDNA using SuperScript II retrotranscription kit (Invitrogen). Quantitative PCR was performed in a 96-well plate by using the SYBR Green Jump Start kit (Sigma). Data were normalized to 36b4 mRNA. For Western blot analysis, cells were lysed in RIPA buffer. Proteins (30 μg) were resolved on 6 or 12% SDS-PAGE, blotted onto nitrocellulose membrane (GE Healthcare), and probed with specific antibodies after saturation. Anti-p21 (sc-397) was from Santa Cruz Biotechnology (Heidelberg, Germany); anti-actin was from Sigma; anti-Rb was from Pharmingen; anti-ERRα was raised in rabbits (15).

**Chromatin Immunoprecipitation**—Cells were incubated for 10 min in phosphate-buffered saline containing 1% formaldehyde. After centrifugation, cell pellets were resuspended in lysis buffer (1% SDS, 50 mM Tris-HCl, pH 8, 10 mM EDTA). Sonication was performed with Bioruptor (Diagenode, Liege, Belgium). Lysates were incubated with 3 μg of antibody and 80 μl of protein A-agarose for 2 h. After washing the immune complexes were eluted from beads in a buffer containing 1% SDS and 0.1 M NaHCO3. Cross-linking was reversed overnight at 65 °C, and DNA fragments were purified using QIAquick column (Qiagen). Quantitative PCRs were performed using 2 μl of DNA in triplicate. Antibodies against Sp1, RNA polymerase II were from Santa Cruz Biotechnology. Primers used for quantitative PCR were as follows: p21 pair A, 5'-GGGGCGGTGTATATCAGGG-3' and 5'-GGCTCAACAGGAACTGTGCT-3'; p21 pair B, 5'-GCTCCAAGCTTGGTTCTC-3' and 5'-CCTTCATTGTGCAGATGTTT-3'; p2 promoter, 5'-GGCCATCTTCTCATAATGACTCTTG-3' and 5'-GCCAGGCTCTGTTTGCTTAAAGAGCCG-3' (taken from Ref. 11).

**RESULTS**

**ERRα Inverse Agonist** XCT790 **Blocks Cell Proliferation and Tumorogenesis**—E2 (10^-8 M) enhanced the proliferation of human mammary carcinoma MCF7 cells. Upon cotreatment with XCT790 (5 x 10^-6 M), proliferation returned to basal level (Fig. 1A). The latter drug also blocked E2-induced BrdUrd incorporation (Fig. 1B). XCT790 exerted a dose-dependent effect on cell proliferation with a half-maximal dose around 5 x 10^-6 M (Fig. 1C), a concentration that we used thereafter. We next tested whether XCT790 could block proliferation in other cell types in which ERRα is expressed (Fig. 1D). The hormone-independent proliferation of mammary carcinoma MDA-MB231 and prostate carcinoma PC3 cells was blocked by XCT790 treatment. As expected LNCaP (prostate carcinoma) cells cultured in the absence of steroid hormones did not proliferate. Epidermal growth factor or testosterone treatment induced proliferation that was inhibited by XCT790 (Fig. 1D and data not shown).

Because XCT790 blocks cell proliferation, we tested whether this compound could inhibit the tumorigenicity of grafted cells in nude mice. To follow tumor growth, we used derivatives of MCF7 cells (MELN cells; see Ref. 32) that were engineered so as to express the luciferase reporter under the control of estrogen response elements (ERE). We first verified that MELN cells still expressed ERRα, are sensitive to XCT790 (in terms of inhibition of proliferation), and that the ERE-driven luciferase activity per se was not modified by XCT790 treatment (data not shown). MELN cells were treated with XCT790 for 24 h before washing and subcutaneous injection in the left flank of mice. Control cells (i.e. vehicle-treated) were grafted contralaterally. 6 weeks after graft, control cells began to form measurable tumors, the volume of which increased with time (Fig. 1E). In contrast, XCT790-treated cells produced measurable tumors only after 10 weeks after which they continuously grew. From week 11 onward, the average size of these tumors was not sig-
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Figure 1. XCT790 inhibits cell proliferation and tumorigenicity. A, after culture for 3 days in the absence of estrogens, MCF7 cells were treated with E2 (10^{-8} M), XCT790 (5 \times 10^{-6} M), or both as indicated. Cell proliferation was estimated after 1, 3, and 5 days. B, same as above counting the number of BrdUrd-positive cells after 24 h of drug treatment. C, E2-stimulated MCF7 cells were treated with the indicated XCT790 concentration. Proliferation was estimated after 5 days. Data are expressed relative to E2-treated controls. D, expression of ERRα protein in the cell lines used in this study is shown in the upper panel (actin is used as a loading control). MDA-MB231 or PC3 cells (lower left and middle panels, respectively) were treated with XCT790, and cell proliferation was estimated after 5 days. Lower right panel, after culture for 3 days in the absence of steroid hormones, LNCaP cells were treated with epidermal growth factor (100 ng/ml), XCT790, or both as indicated, and cell proliferation in the absence of steroid hormones, LNCaP cells were treated with epidermal growth factor (100 ng/ml), XCT790, or both as indicated, and cell proliferation was estimated after 5 days. Experiments were performed in triplicate; data are expressed relative to unstimulated control with error bars representing S.D. E, 2 \times 10^5 MELN cells were treated with XCT790 for 24 h before injection subcutaneously in the right flank of nude mice. Untreated cells were injected contralaterally. Left panel, implanted cells originating luciferase activities were visualized 8 weeks after implantation (a single representative animal is shown). White arrow, untreated cells; black arrowhead, XCT790-treated cells. Right panel, tumor volumes were measured manually at the indicated times after cell graft. All 6 mice (out of 10 grafted) in which tumors were measurable are reported in the graph, which represents mean tumor volume with error bars representing S.E. One-way analysis of variance was performed, comparing control and XCT790-pretreated tumors at each time point. *, p < 0.05; **, p < 0.005; ns, not significant.

Significantly different from control ones, although we observed a nonsignificant trend to being smaller. This indicates that the initial single XCT790 exposure was able to delay tumor growth, a transient effect that is lost with time.

XCT790 Blocks G1/S Phase Transition—Because proliferation of all the cell types tested could be blocked by XCT790 treatment, we chose to focus on MCF7 cells for further mechanistic studies. These cells are indeed a convenient model because proliferation can be switched on by E2 addition. We determined whether XCT790 blocked cells in a specific phase of the cell cycle. As determined by fluorescence-activated cell sorter analysis, E2 induced an expected transition from the G1 to the S phase and allowed the cycle to proceed, with appearance of successive peaks corresponding to S and G2/M phases (Fig. 2 A). In contrast, XCT790 cotreated cells did not enter S phase and stayed in G1 phase at least for 48 h. We next determined whether XCT790 specifically acts in the G1 phase or whether it can block cells in any phase of the cell cycle (Fig. 2B). To this end, G1-arrested cells were induced with E2 and treated 24 h later with XCT790 (i.e. when cells were in S phase peak). Whether treated with XCT790 or not, the same proportion of cells was in G1M at 36 h (after the initial E2 treatment) and could enter G1 at 40 h. At 44 h, cells treated with E2 alone re-entered a second S phase, whereas XCT790-treated cells accumulated in G1. This indicates that cells treated with XCT790 when out of the G1 phase finish their normal cycle and stop in the next G1 phase. In other terms, XCT790 blocks cell cycle specifically in G1. We then tested whether the effect of XCT790 was reversible. To this end, cells were treated for 72 h with XCT790 and, after washing, supplemented with E2. As shown in Fig. 2C, the latter compound was able to promote G1/S transition independently of a prior XCT790 treatment. This indicates that XCT790 displays reversible effects and does not induce cell toxicity. XCT790 has been described to act specifically through ERRα. To verify that this nuclear receptor is indeed involved in the effect of XCT790, cells were transfected with an siRNA that specifically targets ERRα. In these cells, XCT790 was unable to counteract the G1/S transition promoted by E2 (Fig. 2D), indicating that ERRα is required for the effect of XCT790. However, it should be noted that the absence of ERRα itself does not alter the cell capacity to respond to E2. These data thus indicate that XCT790 exerts an active role on ERRα and does more than merely induce the degradation of the receptor.

XCT790 Induces p21 Expression—We next questioned the molecular mechanism through which XCT790 exerts its cell cycle inhibitory effect. We did not observe any clear modification of cyclin D1 expression (data not shown) upon XCT790 exposure. In contrast, the p21 protein was found up-regulated in cells cotreated by E2 and XCT790 in a time-dependent manner as compared with treatment with E2 alone (Fig. 3A). E2 treatment was accompanied by a hyperphosphorylation of Rb, reflecting activation of cyclin-cdk complexes and G1/S transition (Fig. 3B). In contrast, 24 h after XCT790 cotreatment, only hypophosphorylated Rb was detected, which is consistent with an elevation of p21 expression. We next investigated whether the effect of XCT790 was dependent on ERRα (Fig. 3C). To this end cells were transfected with siRNAs directed against the
We next determined the mechanism through which XCT790 regulates p21 protein expression. p21 mRNA level was enhanced by E2/XCT790 cotreatment relative to E2 exposure only (Fig. 4A) suggesting an effect at the transcription level. To determine whether XCT790 enhanced p21 expression at the promoter level, transient transfections were performed in MCF7 cells using derivatives of the human p21 promoter (described in Fig. 4B). A 2.3-kbp promoter fragment was dose-dependently activated by ERRα brought by cotransfection (Fig. 4C). A comparable mouse promoter fragment was also up-regulated by ERRα (data not shown). As already demonstrated (33), expression driven by a shorter derivative (pWP101) of the human p21 promoter was also enhanced by ERRα. Strikingly, treatment with XCT790 enhanced the activating effect of ERRα both on the 2.3-kbp and 101-bp p21 promoter fragments. This is in contrast with the situation observed with the pS2 promoter, an identified target of ERRα in mammary cells (34). Indeed, ERRα dose-dependently activated this promoter, as expected, but this effect was blunted by XCT790 treatment (Fig. 4C). XCT790 can thus act on ERRα in an agonistic or antagonistic manner, depending on the target promoter. Point mutations inactivating the distal Sp1 sites of the pWP101 construct had no consequence on ERRα activation capacity nor on the potentializing effect of XCT790. In contrast, a p21 promoter derivative bearing mutations in the proximal Sp1 sites was unable to support activation by ERRα and XCT790, indicating that these Sp1 sites are required for the effect of the receptor-compound complex. We next investigated the mechanism of these effects by chromatin immunoprecipitation (Fig. 4D). We could not detect any binding of ERRα to the p21 promoter whatever the conditions used, although ERRα bound the pS2 promoter as described (11), a phenomenon that is inhibited by XCT790 exposure. In contrast, RNA polymerase II binding to the p21 promoter was more elevated in the presence of XCT790 confirming that the compound enhanced p21 expression. Binding of Sp1 protein on the p21 promoter (but not on a distal region) was also enhanced by XCT790 treatment. Taken together, we conclude that XCT790 modulates Sp1 binding to
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**FIGURE 3.** XCT790 induces the expression of p21 in an ERRα-dependent, p53-independent manner. A, MCF7 cells were treated with E2 or E2 + XCT790 (E2X), p21 expression was analyzed by Western blotting at the indicated times, using actin as a control. B, Rb phosphorylation was analyzed after 24 h of drug exposure (Rb and P-Rb, hypophosphorylated and hyperphosphorylated Rb isoforms, respectively). C, cells were independently transfected with two ERRα-specific (siE1 and siE2) or control siRNA (siC). Extinction of ERRα (but not that of ERα) expression was verified after 72 h (left panel). Cells were then treated as indicated for 24 h after which p21 expression was determined by Western blotting using actin as a control. D, WT- or p53-deficient MCF7 cells were treated for 24 h with E2 or E2 + XCT790. p21 and p53 expression were analyzed by Western blotting, using actin as a control. Shown are single representative experiments (out of three). E, after culture for 3 days in the absence of estrogens, WT or p53-deficient MCF7 cells were treated by E2 or E2 + XCT790. Percentage of cells in G0/G1 was analyzed after 24 h. Shown is the result of one representative experiment, performed in triplicate. Error bars represent S.D.

The p21 promoter in an ERRα-dependent manner, although without direct detectable binding of the receptor to the promoter.

**DISCUSSION**

In this study, we demonstrate that XCT790, a compound identified as an ERRα inverse agonist, down-regulates cell proliferation. This effect is general as it was observed on hormone-dependent or -independent proliferation as well as growth factor-induced cell growth. This suggests that XCT790 does not interfere with a specific proliferation-inducing pathway and that it could modulate proliferation of any type of cells/tissues expressing ERRα. Inhibition of proliferation does not result from cell mortality due to XCT790. Indeed analysis of the cell cycle distribution did not show any accumulation of sub-G1 cells (supplemental Fig. 1), a typical feature of cellular death. Furthermore, a 72-h treatment with $5 \times 10^{-6} \text{M}$ XCT790 followed by washing did not result in cells floating in the medium, and cell cycle could be resumed upon E2 addition. This is consistent with recent data showing XCT790 toxicity (in HepG2 cells) only when used at concentrations above $10^{-5} \text{M}$ (35).

Consistently, a single XCT790 pretreatment results in delayed tumorigenicity of MCF7 cells in Nude mice, which was observed up to 10 weeks after graft. In contrast no significant difference in tumor volume according to the treatment could be observed after 11 weeks. This indicates that the effect of a single XCT790 treatment is transient, which is consistent with the results obtained in vitro showing that the effect of the drug is reversible (Fig. 2C). In the latter experiment, we observed that an E2 treatment following XCT790 withdrawal immediately overcomes the inhibitory effect of the latter drug. This is also the case in vivo. Indeed, when Nude mice were supplemented with E2 immediately after graft, no effect of the XCT790 pretreatment could be observed (data not shown). It remains to be determined whether XCT790 could induce the regression of a pre-existing tumor. Before this experiment can be performed, however, the in vivo toxicity of XCT790 should be determined.

XCT790 specifically blocks the $G_1/S$ transition. The drug did not lead to p27 protein accumulation, which is characteristic of the $G_1$ phase (36). We thus conclude that XCT790 induces a block in the $G_1$ phase. At least in MCF7 cells, this is accompanied by an induction of p21 expression at the promoter, RNA, and protein levels. As an expected consequence, hypophosphorylated Rb was detected after XCT790 treatment, although this protein was mainly detected as a hyperphosphorylated isoform upon E2 exposure. Importantly, up-regulation of p21 expression by XCT790 as well as its effect on proliferation is p53-independent but ERRα-dependent.

ERRα directly binds on the pS2 promoter through a classical ERRα-response element (11), a binding that is inhibited by XCT790 treatment. We did not detect ERRα (in the presence or absence of XCT790) bound to the p21 promoter, which does not harbor any ERRα-response element. It remains possible that ERRα binds to this promoter in an indirect manner (i.e. through a DNA-bound factor). However, XCT790 induced an enhanced recruitment of Sp1 to its cognate binding sites on the p21 promoter, a phenomenon that may explain the stimulating effect of the compound on p21 expression. The mechanism through which XCT790 exerts this effect is presently unknown, but it could involve the previously demonstrated physical interactions between ERRα and Sp1 (33).

In addition to its inhibitory effects on ERRα, XCT790 can act as an agonist of the receptor, specifically on the p21 promoter. The behavior of XCT790 is thus reminiscent of that of the anti-estrogen ICI182,780 in several features. Indeed, both compounds induce a proteasome-dependent degradation of their cognate receptor (15, 37), repress the expression of the pS2 promoter, and ERRα (in the presence or absence of XCT790) bound to the p21 promoter, which does not harbor any ERRα-response element. It remains possible that ERRα binds to this promoter in an indirect manner (33). However, the main difference is that ERα represses this promoter, and ERRα moderately activates it. ICI182,780 is thus a pure ERα antagonist, whereas XCT790 behaves as an agonist or antagonist according to the target promoter. In agreement with this, elimination of ERRα protein (obtained by siRNA treatment) does not recapitulate the effect of XCT790 on proliferation but leads to decreased p21 expression. However, XCT790 requires the receptor to activate p21 expression and repress proliferation, indicating that its effects are ERRα-specific. Elevation of p21 expression upon XCT790 treatment was also observed on HCT116 cells (human colon carcinoma) but not on MDA-MB231 cells in which no basal p21 expression was observed (data not shown). However, XCT790...
enhanced the expression of the related cell cycle inhibitor p27 in these cells (data not shown). XCT790 may thus block the cell cycle through different, although related, mechanisms.

Recent results have shown that exogenous overexpression of the closely related ERR\textsubscript{H9252}/H9253 in prostate cancer cell lines results in inhibition of proliferation, block in G1 phase, and elevation of p21 expression (38, 39). Furthermore, treatment with an ERR\textsubscript{H9252}/H9253 agonist promotes this anti-proliferative effect. This is consistent with ERR\textsubscript{H9251} being described as a favorable prognosis factor (25). ERR\textsubscript{H9251} on its own also activates the p21 promoter (33 and this study). A high expression of the receptor should thus result in a low proliferation rate. This is in agreement with the observation that terminal differentiation during mouse embryogenesis as well as in different nonmalignant cellular models (C2C12 and 3T3-L1 adipocytes) is accompanied by an elevation of ERR\textsubscript{H9251} expression (40–43). Nevertheless, this is in contradiction with a high ERR\textsubscript{H9251} expression in tumors of various organs correlating with poor prognosis (reviewed in Ref. 29), suggesting that this receptor promotes rather than represses cell proliferation. However, the biological activities of ERR\textsubscript{H9251} may not depend solely on its expression level but could also rely on other events, such as post-translational modifica-

**FIGURE 4.** XCT790 stimulates p21 mRNA expression and promoter activity. A, MCF7 cells were treated by E2 or E2 + XCT790 for the indicated times. p21 mRNA expression was analyzed by quantitative PCR. The experiment was performed in triplicate, and data are expressed relative to unstimulated control with error bars representing S.D. B, schematic representation of the p21 promoter derivatives used. Details of the mutations can be found in Ref. 31. White and black boxes represent wild type and mutant (respectively) Sp1 sites. Arrows represent the position of the primer used in chromatin immunoprecipitation below. C, MCF7 cells were transfected with the indicated promoter constructs together with the indicated amounts of ERR\textsubscript{H9251}-encoding plasmid and treated or not with XCT790 as indicated. pS2 promoter was used as a control. Transfections were performed three times in triplicate and included a CMV-\beta-gal vector. Luciferase activity is expressed relative to \beta-galactosidase activity. For each promoter construct, data are expressed relative to the reporter vector transfected alone and unstimulated. The graphs represent a single experiment performed in triplicate with error bars representing S.D. D, chromatin immunoprecipitation at the p21 (left panel) and pS2 promoters (right panel). MCF7 cells were treated as indicated. Chromatin was immunoprecipitated with the indicated antibodies and submitted to quantitative PCR. Results are expressed as fold enrichment over IgG-immunoprecipitated material. A typical experiment is shown with error bars representing S.D. Pol II, polymerase II.
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tions or the presence of active coregulators. Noteworthy, stimulation of the p21 promoter by ERRα is exacerbated by RIP140 (33). It could be hypothesized that RIP140 not only cooperates with but is actually required for ERRα to activate p21 expression and turn off proliferation. This assumption is consistent with the fact that the expression of both ERRα and RIP140 increases during terminal differentiation of pre-adipocytes with a positive effect of ERRα on the expression of RIP140 (43). This hypothesis also predicts that high expression of ERRα only indicates a poor prognosis when associated with a low expression of RIP140. Validation of this hypothesis will await the determination of the expression of RIP140 in breast tumors and its evaluation as a prognosis factor. If true, this global hypothesis does not document the role of ERRα when RIP140 is absent and may suggest that the latter must be supplemented for ERRα to act as an anti-proliferative factor. However, XCT790 is, in this respect, comparable with RIP140 in that both compounds antagonize ERRα on the pS2 promoter but agonize it on the p21 one. It thus remains possible that a pharmacological treatment targeting ERRα (recapitulating the effects of XCT790) may have beneficial effects on breast cancer even in the absence of RIP140.

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