Potentiation of ICI182,780 (Fulvestrant)-induced Estrogen Receptor-α Degradation by the Estrogen Receptor-related Receptor-α Inverse Agonist XCT790*

Olivia Lanvin†§, Stéphanie Bianco†§, Nathalie Kersual†, Dany Chalbos‡, and Jean-Marc Vanacker†§

From the †Institut de Génomique Fontionnelle, Université de Lyon, F-69003 Lyon, France, ‡Institut National de la Recherche Agronomique (INRA), CNRS, Université Lyon 1, Ecole Normale Supérieure, F-69364 Lyon, France, and §INSERM U826, F-34298 Montpellier, France

ICI182,780 (Fulvestrant) is a pure anti-oestrogen used in adjuvant therapies of breast cancer. This compound not only inhibits the transcriptional activities of the estrogen receptor-α (ERα) but also induces its proteasome-dependent degradation. The latter activity is believed to be required for the antiproliferative effects of ICI182,780. Estrogen receptor-related receptor-α (ERRα) is an orphan member of the nuclear receptor superfamily that is expressed in a wide range of tissues including breast tumors, in which its high expression correlates with poor prognosis. Although not regulated by any natural ligand, ERRα can be deactivated by the synthetic molecule XCT790. Here we demonstrate that this compound also induces a proteasome degradation of ERRα. We also show that although it does not act directly on the steady-state level of ERα, XCT790 potentiates the ICI182,780-induced ERα degradation. We suggest that treatment with XCT790 could thus enhance the efficacy of ICI182,780 in ERα-dependent pathologies such as breast cancer.

Estrogen receptor-α (ERα), a member of the nuclear hormone superfamily, is a ligand-regulated transcription factor that mediates the effects of various estrogenic molecules including 17β-estradiol (E2) (1). Upon interaction with E2, the ligand binding domain (LBD) of ERα adopts an active conformation that allows the recruitment of transcriptional coactivators and the up-modulation of the expression of target genes, on the promoter of which the receptor binds. ERα and its cognate ligands are involved in several physiological processes such as metabolism, regulation of female reproduction, and bone homeostasis (2).

Estrogens are also instrumental in various pathological conditions. For example, breast cancers are often dependent upon estrogens that positively regulate cell proliferation (3). For this reason, molecules that block the transcriptional activities of ERα in the mammary gland were identified (4, 5). These compounds inhibit E2-induced proliferation and some are widely used in adjuvant therapy in breast cancer. This is the case of 4-OH-tamoxifen (OHT), a mixed antagonist (i.e. that displays agonist or antagonist activity depending on the tissue), or ICI182,780 (Fulvestrant), a pure antagonist. Upon interaction with E2, ERα not only becomes transcriptionally active but is also targeted for degradation by the proteasome machinery (6–8). Antagonists affect ERα protein stability in a more complex manner. OHT stabilizes the receptor in an inactive conformation, whereas ICI182,780 induces a rapid degradation of the receptor. Various pathways are identified that contribute to ligand-induced ERα degradation (9–15). Some of these pathways are required for the antiproliferative activities of ICI182,780 (11).

Estrogen receptor-related receptor-α (ERRα) is another member of the nuclear receptor superfamily that is identified on the basis of its high level of sequence identity to ERα (1, 16). ERRα is involved in the regulation of metabolism in cooperation with the PGC-1α coactivator. Indeed, ERRα regulates lipid and glucose metabolism (17–22). The receptor is also essential to the regulation of mitochondrial biogenesis exerted by PGC-1α (23, 24). ERRα is expressed in a wide variety of tissues, and its high expression correlates with poor prognosis in breast, colon, and ovarian cancers (25–29), although its roles in tumors have not been determined.

The transcriptional activities of ERRαs are not regulated by estrogens; however, several levels of interference occur between ERRα and estrogen signaling (30, 31). For example, estrogens regulate the expression of ERRα in mouse uteri (32); in turn ERRα positively regulates the expression of aromatase (33), the limiting enzyme in estrogen biosynthesis. Furthermore, ERα and ERRα display complex positive and negative interactions in the transcriptional regulation of common genes, such as osteopontin, lactoferrin, and TFF1/pS2 (34–38). In vitro far Western experiments show that ERα and ERRα physically interact (39). No natural ligand was identified for ERRα, which is considered an orphan receptor (31).Crystallographic studies show that this receptor spontaneously adopts an active conformation (40). Although ERRα regulates transcription in a
constitutive manner, some of its activities, such as DNA binding or contact with coactivators, can be regulated by phosphorylation (41). Compounds such as the phytoestrogen genistein can inhibit the transcriptional activities of ERα (42). Based on its capacity to disrupt the interaction between ERα and PGC-1α, the synthetic molecule XCT790 is identified as an ERα specific ligand and acts as an inverse agonist (43).

In this report, we show that XCT790 not only represses the transcriptional activities of ERα but also induces it to proteasome-dependent degradation. Although it does not directly affect ERα stability, XCT790 also potentiates the ICI182,780-induced ERα degradation. This effect is dependent on ERα and on new protein synthesis, indicating an indirect effect. Treatment with XCT790 may enhance the efficacy of ICI182,780 in diseases involving ERα such as breast cancer.

**EXPERIMENTAL PROCEDURES**

**Materials**—The following antibodies and reagents were used in this study. Anti-ERα raised in a rabbit, using a keyhole limpet hemocyanin-coupled peptide mapping a region conserved between mouse and human ERα but divergent in other ER subfamily members, was used. Anti-ERα (HC-20) and anti-actin antibodies were purchased from Santa Cruz Biotechnology (Tebu, France) and Sigma-Aldrich, respectively. Actinomycin D, MG132, cycloheximide, XCT790, E2, and OHT were purchased from Sigma-Aldrich, and ICI182,780 was purchased from Tocris Cookson Ltd (Bristol, UK).

**Cell Lines and Culture Conditions**—The human mammary epithelial cell line MCF7 was grown in Dulbecco’s modified Eagle’s medium supplemented with 2 mM l-glutamine, 50 units/ml penicillin, 50 μg/ml streptomycin, and 10% heat-inactivated fetal calf serum. Prior to experiments involving treatment with estrogens, 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. In all experiments except for dose response a 5 μM XCT790 concentration was used.

**Transient Transfection and Luciferase Assay**—Cells (10⁶) were seeded in a 24-well plate in hormone-free medium and transfected using 3 μl of Exgen500 (Euromedex, Soufflawyeyer-sheim, France) with UAS-(Gal4-binding) luciferase reporter constructs, along with Gal4DBD (Gal4) or constructs fusing the Gal4DBD to ERα- or ERα-LBD (50 ng). CMV-βGal plasmid (50 ng) was added to normalize transfection efficiency, and pSG5 plasmid was added as a carrier up to 500 ng. Six hours later, cells were treated or not with XCT790 or E2 (100 nM) for 48 h and then lysed in 200 μl of lysis buffer (125 mM Tris phosphate, pH 7.8, 10 mM EDTA, 5 mM dithiothreitol, 50% glycerol, 5% Triton X-100). Luciferase activity was determined using the luciferase assay system (Promega) and normalized based on β-galactosidase levels. Transfections were performed in triplicate.

**Expression Analysis**—RNAs were isolated by guanidinium thiocyanate/phenol/chloroform extraction. Total RNA was converted to first-strand cDNA using a SuperScript II retrotranscription kit (Invitrogen). Quantitative PCR was performed in a 96-well plate by using the SYBR Green JumpStart kit (Sigma-Aldrich). Data were normalized to 36b4 mRNA. Primers used in this study were: ERα, 5’-CAAGCGGCTCTGCCTG-GTCT-3’ and 5’-ACTCGATGCTCCCCTGGATG-3’; c-Myc, 5’-GCCACGTCTCCACACACTAG-3’ and 5’-TCTGGCAC-GAGGATGTCTCC-3’; hypoxanthine-guanine phosphoribosyltransferase, 5’-CTGACCTGCTGGATTACA-3’ and 5’-GCGACCTTGACCATCTTT-3’; and 36b4, 5’-GTCACT-GTGGAGCCCAGAAA-3’ and 5’-TCAATGGTGCCCCGT-GAGAT-3’.

**RNA Interference (siRNA)**—The ERα (Dharmacon) and ERα (Fisher Invitrogen) siRNAs were transfected into MCF7 cells with Oligofectamine and Lipofectamine RNAiMax, respectively, according to manufacturer’s protocol (Fisher Invitrogen). Cells were transfected twice with ERα or ERα siRNA, first in 10-cm plates for 48 h and then in a 6-well plate for 24 h. Cells were then preincubated with XCT790 or ICI182,780 for 48 h and then treated for 16 h with ICI182,780 or XCT790 depending on the first incubation. In other experiments, cells were transfected with siRNA only once for 48 h and treated with drugs as described above.

**Western Blot Analysis**—For Western blot analysis, MCF7 cells were seeded in 6-well plate (5 × 10⁴) and treated or not with MG132 (4 μM), cycloheximide (10 μg/ml), XCT790, tamoxifen (1 μM), ICI182,780 (1 μM) for the indicated times. Cells were lysed in radioactive immunoprecipitation assay buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 5 mM EDTA, 0.5% Nonidet P-40, 0.1% sodium deoxycholate, 0.1% SDS, and a mixture of protease inhibitors) and then centrifuged for 15 min at 13,200 rpm. Proteins were quantified using the Bradford protein assay kit (Pierce, Perbio Science Co.) and boiled for 10 min in Laemmli buffer. Proteins (30 μg) were resolved on 10% SDS-polyacrylamide gel electrophoresis and blotted onto nitrocellulose membrane (GE Healthcare). The blots were saturated in TBS (50 mM Tris, 150 mM NaCl, 0.1% Tween) containing 5% nonfat dry milk and then probed with specific antibodies (anti-ERα, anti-ERα, anti-actin) and developed using an enhanced chemiluminescence detection system (ECL kit, GE Healthcare) with appropriate specific peroxidase-conjugated donkey anti-rabbit antibody (GE Healthcare).

**RESULTS**

**XCT790 Induces ERα Protein Degradation**—To verify that XCT790, an ERα inverse agonist, specifically inactivated this receptor in human mammary cells, an UAS-Luc reporter plasmid was transfected into MCF7 cells with a Gal4-ERα-LBD fusion plasmid. XCT790 down-regulated the activation driven by the latter construct in a dose-dependent manner, but not in the manner exerted by a Gal4-ERα-LBD plasmid (Fig. 1A). We analyzed the effect of XCT790 on ERα protein expression. As shown by Western blot, XCT790 reduced the amount of ERα protein in a dose- (Fig. 1B) and time-dependent (Fig. 1C) manner. This effect of XCT790 was transient because the expression of ERα was restored to its original level 8 h after withdrawal of the drug (Fig. 1D).

We established whether XCT790 exerted its effect on ERα expression at the RNA or protein level. As determined by quantitative PCR, no reduction in the steady-state level of ERα mRNA was evidenced upon XCT790 treatment even after 48 h (Fig. 2A, left panel). It has been reported that expression of the
ERRα gene can be enhanced by its own product (44). A reduction of ERRα protein level is expected to result in a drop of ERRα mRNA expression, which could be masked by a high stability of ERRα mRNA. However, when cells were treated with actinomycin D, a global transcriptional inhibitor, the steady-state level of ERRα mRNA was rapidly reduced (Fig. 2A, right panel). The half-life of this mRNA was clearly shorter than 2 h, comparable with that of c-Myc (used as an unstable mRNA control) and well beyond that of hypoxanthine-guanine phosphoribosyltransferase (used as a stable mRNA control). ERR control) and well beyond that of hypoxanthine-guanine phosphoribosyltransferase (used as a stable mRNA control). ERR mRNA was measured by quantitative PCR and normalized to the level of 36B4 mRNA (right panel). ERR mRNA is unstable. RNA was extracted after the indicated time of actinomycin D treatment. ERR mRNA level was measured using specific primers in quantitative PCR. The experiments were performed twice in triplicate. Error bars indicate standard deviation. A, XCT790 acts on ERR protein in a proteasome-dependent manner. Cells were treated for 16 h with XCT790 and/or MG132 (a proteasome inhibitor) and processed for Western blot analysis. B, XCT790 accelerates ERR protein turnover. MCF7 cells were treated for 16 h with XCT790 and/or cycloheximide (CHX, a protein synthesis inhibitor) and processed for Western blot.

XCT790 Effects on Antagonist-induced ERRα Degradation—We next addressed the specificity of action of XCT790. When used alone the drug reduced the expression of ERRα protein but had no effect on ERRα (Fig. 3A, left panel). As expected, a 16 h treatment with ICI182,780, an ERα antagonist, reduced the protein expression of ERRα, but not ERRα. Strikingly, exposure to both molecules led to a complete disappearance of ERRα signal, indicating that XCT790, inactive per se on ERRα, greatly potentiated the degradation-inducing effect of ICI182,780. XCT790 acted at the protein level because the steady-state level of ERRα mRNA was not modulated (Fig. 3A, right panel). In contrast ICI182,780 treatment was accompanied by an up-modulation of ERRα mRNA level, an expected result because ERRα is autoregulated in MCF7 cells (45). We next tested the possibility of the reverse effect, i.e. an effect of ICI182,780 on XCT790-induced ERRα degradation (Fig. 3B). To this end, we modified the order and length of exposure to the drugs. After 48 h, ICI182,780 reduced the expression of ERRα protein but not that of ERRα (Fig. 3B, left panel). XCT790 induced a moderate reduction of ERRα expression, an effect that was dramatically enhanced upon ICI182,780 treatment. Again this potentiation effect was at the protein, but not the mRNA, steady-state level
We concluded that XCT790 and ICI182,780 potentiate the degradation-inducing effect of one another in addition to their direct action on their cognate receptor. In contrast to ICI182,780, OHT, another ERα-antagonist in MCF7 cells, stabilizes ERα, thus leading to an elevation of the steady-state level of the receptor (7). We examined the relationships between OHT and XCT790 (Fig. 3C). Pretreatment with the latter drug did not enhance or reverse the stabilizing effect of OHT on ERα (Fig. 3C, left panel). Similarly, OHT did not enhance the XCT790-induced degradation of ERα (Fig. 3C, right panel).

**XCT790 Acts Indirectly on ERα Stability in an ERα-dependent Manner**—XCT790 acts on both the transcriptional activity and expression of ERα protein. It is possible that the modulation of antagonist-induced ERα degradation by XCT790 is because of the absence of ERRα receptor. If so, knocking down ERRα should also result in a potentiation of the ERα-degrading effect of ICI182,780. Transfection of an siRNA directed against ERRα resulted in a nearly complete elimination of the receptor (Fig. 4A, left panel). However, under these conditions ICI182,780 did not display any enhanced ERα-degrading effect as compared with cells transfected with a control siRNA. This indicates that the indirect effect of XCT790 on ERα stability is not simply a consequence of ERRα disappearance. This result also questions the requirement of ERRα in XCT790 effect on ICI182,780-induced ERα degradation. Cells were thus transfected with ERRα-directed siRNA and subsequently treated with XCT790 (Fig. 4A, right panel). Under these conditions, the drug was unable to enhance ICI182,780-induced ERα degradation, indicating that ERRα is absolutely required for the effect of XCT790. We also tested whether the absence of ERα resulted in an enhanced ERRα degradation effect of XCT790. XCT790 was not more potent in inducing ERRα degradation in cells treated with an ERα-targeting siRNA than in siRNA-treated controls (Fig. 4B, left panel). However, ICI182,780 absolutely required ERα to modulate XCT790-induced ERRα degradation (Fig. 4B, right panel).

**Cross-modulation of ERRα and ERα Stability Requires New Protein Synthesis**—The above results show that receptor absence and treatment by antagonist drugs are not equivalent. Furthermore, a pretreatment by a given drug is required to enhance the capacity of the other drug to degrade its cognate receptor (i.e. a cotreatment is inefficient for this promotion, data not shown). This 48-h pretreatment (Fig. 3) could be reduced to 12 h (Fig. 5). This suggests an active role of the drugs in modulating the expression of an intermediate factor involved in receptor stability. To evaluate this possibility, cells were treated with cycloheximide, subsequently with XCT790, and then with ICI182,780 (Fig. 5A). Under these conditions, XCT790 did not up-modulate ERα sensitivity to ICI182,780, indicating that new protein synthesis was required for the indirect effect of XCT790 on ERα. We also performed the converse experiment in which cells were treated with cycloheximide, subsequently with ICI182,780, and then with XCT790 (Fig. 5B). Our results show

**FIGURE 3. Interference between ERRα and ERα through degradation control.** Experimental strategy is depicted on each figure part. A, impact of XCT790 on ICI182,780-induced ERα degradation. Cells were treated with XCT790 and/or ICI182,780 as indicated on the upper left panel. Cells were lysed and processed for Western blot (lower left panel) or mRNA quantitation using quantitative PCR (right panel). B, same as A, using the protocol depicted on upper left panel. C, same as A using OHT treatment and Western blot experiment.
that ICI182,780 required the new protein synthesis of an intermediate protein to up-modulate ERRα sensitivity to XCT790.

**DISCUSSION**

**XCT790 as an ERRα Degradation-inducing Agent**—XCT790, a synthetic compound identified on the basis of its capacity to disrupt the interactions between ERRα and the PGC-1α coactivator, down-regulates the constitutive transcriptional activity of ERRα (43). The effect has been observed in CV-1 and also in COS1 cells (46). This observation can be extended to other cell types: XCT790 acts as a powerful ERRα inverse agonist in MCF7 and MDA-MB231 cells (this study and data not shown). XCT790 promotes the degradation of ERRα protein. Expression of the corresponding mRNA is unaffected. Induction of cognate receptor protein degradation, independent of any effect on the corresponding mRNA, was demonstrated for other NR/ligand pair such as ERα/ICI182,780 or PPARγ/pioglitazone (47–49). The ERRα mRNA is very unstable (half-life < 2 h), which is consistent with the circadian regulation of its expression in vivo (50). In contrast the ERRα protein is very stable, as its half-life is estimated to be over 24 h based on cycloheximide treatment (data not shown). The ERRα gene is positively auto-regulated (41, 44). Lowering the level of ERRα protein could result in reduction of the level of the corresponding mRNA, a phenomenon we could not observe. However, if ERRα clearly binds to its own promoter in the absence of any given stimulus (41), activation of the promoter is only seen in the presence of PGC-1α, a factor that we could not detect in the MCF7 strain we used (data not shown). Another nonexclusive hypothesis is that ERRα activity on its own promoter requires additional factors or stimuli. XCT790 requires the proteasome, but no new protein synthesis, to induce the degradation of ERRα, but the precise mechanism of this degradation remains to be determined. XCT790-induced degradation also requires the N-terminal domain of ERRα, because a deletion mutant lacking this part of the receptor is resistant to this effect (data not shown). It could be hypothesized that the phosphorylation events that occur in this region of the receptor (41) may be involved in regulating the stability of ERRα. However, blocking various phosphorylation pathways such as mitogen-activated protein kinase, phosphatidylinositol 3-kinase/Akt, or PKCδ did not alter the potency of XCT790 (data not shown). The effect of XCT790 on ERRα is comparable with that of ICI182,780 on ERα in that each compound blocks the transcriptional effect of its cognate receptor and induces its proteasome-dependent degradation (7). It is distinct from the effect of OHT, a mixed ERα antagonist that stabilizes ERα.

**A New Level of Cross-talk between ERα and ERRα**—Previous research shows that XCT790 specifically targets ERRα (43). Consistently, XCT790 did not exert a direct effect on ERα either by modulating transcriptional activity or by modifying its stability. On the contrary, XCT790 potentiated the degradation-inducing effect of ICI182,780 on its cognate receptor in a strictly ERRα-dependent manner. Far Western
experiments show that ERRα and ERα could physically interact with each other in vitro (39). It could be that these receptors heterodimerize in vivo and that these contacts could “protect” each other from antagonist-induced degradation. Eliminating one receptor by a degradation-inducing antagonist would result in oversensitivity of the other receptor to its own antagonist. In this respect, we noted that ERα is more sensitive to XCT790 in MDA-MB231 (ERα-deficient cells) than in MCF7 (ERα-positive cells) (data not shown). However, restoration of ERα expression in the former cells did not yield a reduced sensitivity of ERα to XCT790. Furthermore, treatment of MCF7 cells with a siRNA directed against one receptor does not result in enhanced sensitivity of the other receptor to its antagonist (Fig. 4).

The above hypothesis assumes that ERα and ERα heterodimerize, a phenomenon that we could not observe by gluthathione S-transferase pull-down or by communoprecipitation in vivo (data not shown). Altogether, this suggests that XCT790 plays an indirect but active role (i.e. more than through inducing ERRα degradation) in enhancing the effect of ICI182,780 on ERα degradation. Consistent with this, the effect of XCT790 could only be observed after a minimum 12-h pretreatment time and not upon cotreatment, suggesting additional factors must be produced. This hypothesis was confirmed when cycloheximide treatment blocked the enhancing effect of XCT790. We tested the effect of XCT790 on the expression of factors involved in proteasome-dependent ICI182,780-induced ERα degradation (6, 8–11, 13, 51). No response to XCT790 was observed for Uba3 (ubiquitin-activating enzyme 3, the catalytic subunit on the NEDD8 pathway), E6AP (an E3 ubiquitin ligase), or UbcH7 (an ubiquitin-conjugating enzyme) (data not shown). The potentiation of XCT790-induced degradation by ICI182,780 also requires both ERα and new protein synthesis of an unknown factor. ICI182,780 and XCT790 behaved in a similar manner, although it is not known whether the intermediate factor required is identical in both cases. XCT790 potentiates the degradation induced by ICI182,780, but not the degradation induced by E2, the natural agonist of ERα (data not shown). This phenomenon is, however, not intrinsically linked to the antagonist nature of ICI182,780. OHT, which behaves as an ERα antagonist in MCF7 cells, induces ERα stabilization, and this effect could not be reverted by XCT790. ICI182,780 treatment leads to the inhibition of ERα-dependent transcriptional activity. Pretreatment with XCT790 did not enhance this blocking effect (data not shown), indicating that the effect of ICI182,780 on transcription can be separated from that on ERα degradation with only the latter being targeted by XCT790 through ERRα. Our results indicate a new level of interference between ERα and ERRα, in that a destabilizing antagonist to one receptor modulates the degradation of the other induced by a cognate antagonist, although the precise mechanism by which they do so remains to be determined.

Possible Consequences in Anti-estrogenic Therapies—ICI182,780 is used in neoadjuvant therapy of breast cancer because it blocks estrogen-dependent cell proliferation. In MCF7 cells, blocking the NEDD8 pathway, an essential component of ICI182,780-induced ERα degradation, reduces the antiproliferative efficacy of the drug, suggesting that receptor degradation is an integral part of the antiproliferative effect (11). It can be expected that treatment with XCT790, as potentiating the degradation of ERα induced by ICI182,780, could also enhance the antiproliferative effect of the anti-estrogen. However, in MCF7 cells, pretreatment with XCT790 did not potentiate the negative effect of ICI182,780 on proliferation. Each ERα moiety contacted by ICI182,780 is transcriptionally blocked immediately and rapidly degraded by the proteasome. This “all-or-nothing” approach may not be effective in vivo, particularly with patients with intact tumors. The kinetics of ERα occupancy by ICI182,780 might not be identical in cells and in vivo. A high ERα expression in breast tumors correlates with poor prognosis. An inverse correlation between ERα and ERRα has been suggested (25) but not confirmed in a larger screen (26). Tumors should thus exist that express both ERα and ERRα. For these tumors, our data suggest that pretreatment with XCT790 could induce a component involved in ICI182,780-induced ERα degradation and thereby enhance the efficacy of the latter drug and reduce its efficient dose.

Acknowledgment—We thank Vincent Cavaillé for helpful discussions.

REFERENCES

1. Laudet, V., and Gronemeyer, H. (2002) The Nuclear Receptor Factbook, Academic Press, San Diego, CA
2. Barkhem, T., Nilsson, S., and Gustafsson, J. A. (2004) Am. J. Pharmacol. 9607–9615
3. Ariazi, E. A., Ariazi, J. L., Cordera, F., and Jordan, V. C. (2006) Curr. Top. Med. Chem. 6, 181–202
4. Osborne, C. K. (1998) N. Engl. J. Med. 339, 1609–1618
5. Ali, S., and Coombes, R. C. (2002) J. Biol. Chem. 373, 978–1985
6. Nawaz, Z., Lonard, D. M., Dennis, A. P., Smith, C. L., and O'Malley, B. W. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 1858–1862
7. Wijayaratne, A. L., and McDonnell, D. P. (2001) J. Biol. Chem. 276, 35684–35692
8. Calligé, M., and Richard-Foy, H. (2006) Nucl. Recept. Signal. 4, e004
9. Lonard, D. M., Nawaz, Z., Smith, C. L., and O'Malley, B. W. (2000) Mol. Cell. 5, 939–948
10. Fan, M., Long, X., Bailey, I. A., Reed, C. A., Osborne, E., Gize, E. A., Kirk, E. A., Bigsby, R. M., and Nephew, K. P. (2002) Mol. Endocrinol. 16, 315–330
11. Fan, M., Bigsby, R. M., and Nephew, K. P. (2003) Mol. Endocrinol. 17, 356–365
12. Calligé, M., Kieffer, I., and Richard-Foy, H. (2005) Mol. Cell. 25, 4349–4358
13. Li, L., Li, Z., Howley, P. M., and Sacks, D. B. (2006) J. Biol. Chem. 281, 1978–1985
14. Zhang, H., Sun, L., Liang, J., Yu, W., Zhang, Y., Wang, Y., Chen, Y., Li, R., Sun, X., and Shang, Y. (2006) EMBO J. 25, 4223–4233
15. Long, X., and Nephew, K. P. J. Biol. Chem. 281, 9067–9075
16. Girgure, V., Yang, N., Segui, P., and Evans, R. M. (1988) Nature 331, 91–94
17. Luo, J., Sladek, R., Carrier, J., Bader, J. A., Richard, D., and Girgure, V. (2003) Mol. Cell. 23, 7947–7956
18. Schreiber, S. N., Knutti, D., Brogili, K., Uhlmann, T., and Kralli, A. (2003) J. Biol. Chem. 278, 9013–9018
19. Carrier, J. C., Deblois, G., Champigny, C., Levy, E., and Girgure, V. (2004) J. Biol. Chem. 279, 52025–52058
20. Huss, I. M., Torra, I. P., Staels, B., Girgure, V., and Kelly, D. P. (2004) Mol. Cell. 24, 9079–9091
21. Wendé, A. R., Huss, I. M., Schaeffer, P. J., Girgure, V., and Kelly, D. P. (2005) Mol. Cell. 25, 10684–10694
22. Herzog, B., Cardenas, J., Hall, R. K., Villena, J. A., Budge, P. J., Girgure, V.,...
Potentiation of ERα Degradation by XCT790

Granner, D. K., and Kralli, A. (2006) J. Biol. Chem. 281, 99–106

23. Schreiber, S. N., Emter, R., Hock, M. B., Knutti, D., Cardenas, J., Podvinec, M., Oakeley, E. I., and Kralli, A. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 6472–6477

24. Villena, J. A., Hock, M. B., Chang, W. Y., Barcas, J. E., Giguère, V., and Kralli, A. (2007) Proc. Natl. Acad. Sci. U. S. A. 104, 1418–1423

25. Ariazi, E. A., Clark, G. M., and Mertz, J. E. (2002) Cancer Res. 62, 6510–6518

26. Suzuki, T., Miki, Y., Moriya, T., Shimada, N., Ishida, T., Hirakawa, H., Ohuchi, N., and Sasano, H. (2004) Cancer Res. 64, 4670–4676

27. Cavallini, A., Notarnicola, M., Giannini, R., Montemurro, S., Lorusso, D., Visconti, A., Minervini, F., and Caruso, M. G. (2005) Eur. J. Cancer 41, 1487–1494

28. Sun, P., Sehouli, J., Denkert, C., Mustea, A., Könsger, D., Koch, I., Oskay-Ozcelik, G., Wei, L., and Lichtenegger, W. (2005) J. Mol. Med. 83, 457–467

29. Ariazi, E. A., and Jordan, V. C. (2006) Curr. Top. Med. Chem. 6, 203–215

30. Giguère, V. (2002) Trends Endocrinol. Metab. 13, 220–225

31. Horard, B., and Vanacker, J. M. (2003) J. Mol. Endocrinol. 31, 349–357

32. Shigeta, H., Zuo, W., Yang, N., DiAugustine, R., and Teng C. T. (1997) J. Mol. Endocrinol. 19, 299–309

33. Yang, C., Zhou, D., and Chen, S. (1998) Cancer Res. 58, 5695–5700

34. Zhang, Z., and Teng, C. T. (2000) J. Biol. Chem. 275, 20837–20846

35. Lu, D., Kiriyama, Y., Lee, K. Y., and Giguère, V. (2001) Cancer Res. 61, 6755–6761

36. Kraus, R. J., Ariazi, E. A., Farrell, M. L., and Mertz, J. E. (2002) J. Biol. Chem. 277, 24826–24834

37. Vanacker, J.-M., Pettersson, K., Gustafsson, J. A., and Laudet, V. (1999) EMBO J. 18, 4270–4279

38. Vanacker, J.-M., Delmarre, C., Guo, X., and Laudet, V. (1998) Cell Growth Differ. 9, 1007–1014

39. Yang, N., Shigeta, H., Shi, H., and Teng, C. T. (1996) J. Biol. Chem. 271, 5795–5804

40. Kallen, J., Schlaeppi, J. M., Bitsch, F., Filipuzzi, I., Schilb, A., Riou, V., Graham, A., Strauss, A., Geiser, M., and Fournier, B. (2004) J. Biol. Chem. 279, 49330–49337

41. Barry, J. B., and Giguère, V. (2005) Cancer Res. 65, 6120–6129

42. Suetsugi, M., Su, L., Karlsberg, K., Yuan, Y., and Chen, S. (2003) Mol. Cancer Res. 1, 981–991

43. Willy, P. J., Murray, I. R., Qian, J., Busch, B. B., Stevens, W. C., Martin, R., Mohan, R., Zhou, S., Ordentlich, P., Wei, P., Sapp, D. W., Horlick, R. A., Heymann, R. A., and Schulman, I. G. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 8912–8917

44. Laganière, J., Tremblay, G. B., Dufour, C. R., Giroux, S., Rousseau, F., and Giguère, V. (2004) J. Biol. Chem. 279, 18504–18510

45. Donahue, C., Westley, B. R., and May, F. E. (1999) Mol. Endocrinol. 13, 1934–1950

46. Nichol, D., Christian, M., Steel, J. H., White, R., and Parker, M. G. (2006) J. Biol. Chem. 281, 32140–32147

47. Alarid, E. T., Bakopoulos, N., and Solodin, N. (1999) Mol. Endocrinol. 13, 1522–1534

48. Hauser, S., Adelmant, A., Sarraf, P., Wright, H. M., Mueller, E., and Spiegelman, B. M. (2000) J. Biol. Chem. 275, 18527–18533

49. Alarid, E. T. (2006) Mol. Endocrinol. 20, 1972–1981

50. Horard, B., Rayet, B., Trigueneaux, G., Laude, V., Delaunay, F., and Vanacker, J.-M. (2004) J. Mol. Endocrinol. 33, 87–97

51. Verma, S., Ismail, A., Gao, X., Fu, G., Li, X., O’Malley, B. W., and Nawaz, Z. (2004) Mol. Cell. Biol. 24, 8716–8726