Histone deacetylase inhibition and estrogen receptor α levels modulate the transcriptional activity of partial antiestrogens

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Abstract

In this study, we have analysed the effects of histone deacetylase (HDAC) inhibition on estrogen receptor (ER) expression and on its transcriptional activity in response to antiestrogens. In several breast cancer cell lines, trichostatin A (TSA), a potent HDAC inhibitor, strongly decreases ER expression in a dose-dependent manner. This repression is observed independently of the presence of ligand and also occurs in ovarian and endometrial cell lines. In addition, we show that in MCF7 cells bearing a stably transfected reporter plasmid (MELN cells), partial antiestrogens such as 4-OH-tamoxifen (OHTam), raloxifen or LY117018, switch to an agonist activity upon HDAC inhibition. This effect is blocked by the pure antiestrogen ICI182780 and exhibits a half-maximal concentration of OHTam equivalent to its affinity for ER. The TSA-dependent decrease of ER expression is required to induce the agonist switch of OHTam properties as it is lost in cells constitutively expressing exogenous receptors (MELN-ER or ER). By contrast, the transrepression activity of OHTam is abolished by TSA independently of the decrease of ER expression. Interestingly, in MELN-ER, ICI182780 remains inhibitory suggesting the involvement of HDAC-independent mechanisms. Finally, in the absence of TSA, transcriptional activity in response to OHTam is significantly raised in MELN cells expressing low levels of ER after transfection of antisense oligonucleotides. In conclusion, inhibition of HDAC enzymatic activity and modulation of ER levels tightly control the relative agonist activity of partial antiestrogens on a stably integrated reporter transgene.


Introduction

Estrogens are important regulators of gene expression in target tissues such as the mammary gland or the uterus where they control the growth of both normal and cancer cells (Topper & Freeman 1980). These effects are mediated by specific estrogen receptors (ER) α and β (Katzenellenbogen & Korach 1997) which belong to a superfamily of nuclear receptors that function as ligand-dependent transcription factors. Transcription is mediated by means of two activation functions (AF) acting synergistically, AF1 located in the N-terminal domain and AF2 in the hormone-binding domain. The AF1 function is constitutively active whereas AF2 requires the presence of hormone and is inactivated by antiestrogens. The strength of each activation domain together with their synergistic action varies depending on the cell and promoter types. Determination of the crystal structures of the ER ligand-binding domain with agonist and antagonist ligands revealed the conformational changes that modulate the interaction with transcriptional coactivators (for a review see Bourguet et al. 2000).

The best-characterized complex includes proteins from the p160s family (Leo & Chen 2000) which in turn recruit CBP/p300 (Kamei et al. 1996, Smith et al. 1996,) and p/CAF (Yang et al. 1996). All these proteins have been shown to possess intrinsic histone acetyltransferase (HAT) activities capable of acetylating histones (Roth et al. 2001) as
well as non-histone substrates such as nuclear receptors (Fu et al. 2000, Jacob et al. 2001, Wang et al. 2001) and transcription cofactors (Chen et al. 1999, Vo et al. 2001). When histone tails are acetylated, the nucleosomal structure appears to be destabilized thus generating a transcriptionally favorable environment allowing transcription factor access to nucleosomal DNA. However, acetylation is a dynamic post-translational modification and the regulation of its steady state level also implicates the opposing activities of histone deacetylases (HDACs). Several reversible or irreversible HDACs inhibitors have been characterized and these compounds, which modify the balance between HAT and HDAC activities, are able to induce histone hyperacetylation and to regulate gene expression. Interestingly, HDAC inhibition has been associated in some cases with a decrease of gene expression, as previously reported for the ERα gene in response to sodium butyrate (deFazio et al. 1992).

Antiestrogens such as tamoxifen, which is the drug most commonly used in endocrine therapy to block estrogen action (O'Regan & Jordan 2002), exhibit mixed estrogenic and antiestrogenic activities depending on the species, tissue, cell and promoter context (Tzukerman et al. 1994). In the presence of 4-OH-tamoxifen (OHTam), ERα has been shown to interact with the N-CoR corepressor and this binding is decreased by intracellular pathways which switch OHTam from an antagonist to an agonist function (in response for instance to forskolin or growth factors) (Lavinsky et al. 1998). The relevance of these interactions is supported by transient transfection experiments showing that overexpression of coactivators (SRC-1 or L7/SPA) or corepressors (N-CoR or SMRT) control the activity of antihormones (Jackson et al. 1997, Zhang et al. 1998, Liu et al. 2002).

Generally, corepressors mediate transriptional repression by recruiting HDACs in a Sin3-dependent or -independent pathway (Huang et al. 2000). In humans, 11 HDACs have been identified to date and are classified in three classes: class I (HDACs 1–3 and 8), class II (HDACs 4–7, 9 and 10) and class III (seven human proteins homolog to yeast SIR2 enzyme) (Muscat et al. 1998, Khochbin et al. 2001). Recent data obtained using the chromatin immunoprecipitation technique have demonstrated that both SMRT and N-CoR together with a subset of HDACs are indeed recruited in the presence of OHTam on ER-regulated target genes such as pS2 or cathepsin D (Shang et al. 2000, Metivier et al. 2002).

The aim of this study was to characterize better the role of acetylation in estrogen signalling. We first show that HDAC inhibition decreases ERα protein accumulation in all the cell lines tested. Using MCF7 cells stably transfected with an estrogen response element (ERE)-containing luciferase reporter plasmid, we demonstrate that HDAC inhibitors such as trichostatin A (TSA) not only abolish the transrepression ability of partial antiestrogens but also increase their agonist activity, the latter effect involving a mechanism dependent on the reduction of ERα expression.

**Materials and methods**

**Plasmids, oligonucleotide and reagents**

The ERE-βGlob-Luc-SVneo plasmid has been described elsewhere (Balaguer et al. 1999). The ERα expression vectors (HEGO, HE15, HE19 and AF2 mut) were given by P Chambon (IGBMC, Strasbourg, France). The expression vectors for human ERα or ERβ (puromycin resistance) were as described previously (Balaguer et al. 2001). The Kmut plasmid encoding the ERα with lysines K302 and K303 replaced by alanine was generated using QuickChange (Stratagene, Amsterdam, The Netherlands) according to the manufacturer’s instructions. The phosphorothioate antisense oligonucleotide (5'-TCATGGTACATGGTCCGT-3') covering the ATG of human ERα cDNA was purchased from Eurogentec (Seraing, Belgium). The FR901228 compound was obtained from Dr M Weber (LBME, Toulouse, France). TSA, puromycin, actinomycin D and cycloheximide were from Sigma.

**Cell culture**

The MELN cell line is derived from MCF7 cells which were stably transfected with the ERE-βGlob-Luc-SVneo plasmid (Balaguer et al. 2001). Monolayer cell cultures (MCF7, PEO-4, OVCAR, Ishikawa, T-47D, ZR-75 and CAMA-1) were grown in Ham’s F-12/Dulbecco’s modified Eagle’s medium (1:1) (F12/DMEM) supplemented with 10% fetal calf serum (FCS) (Invitrogen) and antibiotics. Before hormonal treatments, cells were
stripped of endogenous steroids by passage in medium without phenol red containing 3% charcoal-stripped FCS (FCS/DCC). Control cells were grown under the same conditions and complemented with vehicle alone (ethanol).

**Generation of MELN double-stable transfectants**

Stable transfectants were obtained as previously described (Balaguer et al. 2001). Briefly, to obtain MELN-ER or β, the parental MELN cell line was transfected with plasmids conferring resistance to puromycin and encoding either ERα, ERβ-Flag or empty pSG5 as a control. The MELN-ER clones were selected by measuring, on TSA-treated cells, the expression of ER (α or β) using a ligand-binding assay. In parallel, several mock-transfected clones were randomly selected.

**Western blot analysis and estrogen receptor enzyme immuno assay (EREIA)**

Whole-cell extracts were prepared in 0.4 M KCl, 20 mM HEPES (pH 7.4), 20% glycerol, 1 mM dithiothreitol and proteases inhibitors. Proteins were quantified using the Bradford assay (Bio-Rad Laboratories, Marnes, France). For Western blot, 60 µg were usually loaded on SDS-PAGE and transferred to polyvinyl difluoride (PVDF) membrane. The blots were saturated in TBST buffer (50 mM Tris, 150 mM NaCl, 0.1% Tween 20 (v/v), 5% dehydrated milk (w/v)), incubated with specific primary antibodies for ERα (Tebu, Le Perray, France), ERβ (UCG40, gift of Dr Greene, University of Chicago, USA) or actin (Sigma) and with the appropriate second antibody (Sigma). Detection was carried out using the Chemiluminescence Reagent Plus kit (PerkinElmer Life Science, Courtaboeuf, France). Estrogen Receptor Enzyme Immuno Assay (EREIA; Abbott Laboratories, Rungis, France) was performed according to the manufacturer’s instructions.

**Transient transfection and luciferase assays**

For transient transfection experiments, MELN cells were plated at about 90% confluence into 24-well plates (3 × 10^5 cells) and transfected using Lipo- fectamin 2000 (Invitrogen) according to the manufacturer’s conditions (2 µl reagent and 1 µg DNA per well). Cell extract preparation was carried out as recommended by Promega and luciferase activity was measured on 100 µl of supernatant. Data were expressed as means ± s.d. When appropriate, statistical analyses were performed using the independent samples t-test.

**Results**

*ERα expression is decreased in response to HDAC inhibition*

By Western blot analysis, we first analyzed the effects of HDAC inhibition on ERα expression and showed that, in MCF7 cells, the accumulation of ERα protein was markedly repressed by TSA, a potent and reversible HDAC inhibitor (Fig. 1A). This effect was stronger than the previously described regulation by 17-estradiol (E2) and was independent of the presence or absence of ligands. A similar regulation was obtained with different HDAC inhibitors, structurally related or not to TSA, such as FR901228, suberoylanilide hydroxamic acid (SAHA), HC-toxin or valproic acid (not shown). These results thus confirmed previous data obtained using sodium butyrate, another HDAC inhibitor with lower efficiency and specificity (deFazio et al. 1992). Moreover, we showed that the repression upon TSA treatment was not restricted to MCF7 cells since, as shown in Fig. 1B and C, the same inhibition occurred in ERα-positive ovarian (PEO4) and endometrial (Ishikawa) cells and in three other breast cancer cell lines (T-47D, ZR75–1 and CAMA-1). In order to quantify more precisely ERα protein accumulation, we used the EREIA enzymatic assay and showed in dose–response experiments that, depending on the cell line, the repression was maximal between 100 and 300 ng/ml TSA (Fig. 1C).

*HDACs inhibitors increase OHTam activity on a stably integrated reporter*

To determine if part of gene regulation by antiestrogens was due to HDAC activity, we investigated, in MCF7 breast cancer cells, the effects of TSA on ER transactivation in response to OHTam. Since the chromatin structure may not be completely organized and compacted on transiently transfected DNA (Jeong & Stein 1994), the effect of HDAC inhibition was studied on a reporter gene stably integrated in the genome. The
MELN cells were derived from MCF7 cells after stable integration of the ERE-αGlob-Luc-SVneo plasmid containing the αGlob promoter under the control of an ERE (Balaguer et al. 1999). In this model (Fig. 2A), E2 treatment increased by more than 5-fold the luciferase activity whereas OHTam exhibited a reproducible transrepression activity (2- to 3-fold under the control value) (Balaguer et al. 1999). As previously described in similar models (Bartsch et al. 1996, Minucci et al. 1997), inhibition of HDAC activity by TSA in MELN cells resulted in a ligand-independent increase of the reporter activity (around 20-fold). More interestingly, specific variations occurred in ligand-induced transcriptional responses (Fig. 2A). Treatment of cells with TSA at a concentration of 100 ng/ml completely abolished the OHTam transrepressive effect. At higher concentrations of TSA (500 ng/ml), the antiestrogen presented a clear agonist activity (2- to 3-fold above the value in the absence of ligands) and could become even more potent than estradiol in inducing reporter gene expression. In relation to this observation, we found that TSA concentrations around 500 ng/ml were also required for maximal acetylation of bulk histones in MELN cells (data not shown). Moreover, in the literature, high concentrations of TSA (up to 1·5 µg/ml) appeared to be necessary for maximal HDAC inhibition and subsequent regulation of gene expression (Chen et al. 1997, Mao & Shapiro 2000) or protein acetylation (Ito et al. 2001).

The effect of TSA on OHTam agonist activity was observed in several independent clones of MELN cells, thus suggesting that the TSA effect did not rely on genomic positioning. Moreover, the FR901228 compound, which is an irreversible HDAC inhibitor structurally unrelated to TSA (Nakajima et al. 1998), gave similar results (Fig. 2A). As expected from its higher potency, the FR901228 molecule induced OHTam agonist activity at lower concentrations than those required for TSA. Furthermore, when MELN cells were treated with SAHA, OHTam also exhibited a clear agonist activity whereas the basal transcription of the luciferase reporter was unaffected, thus suggesting that the two effects may involve different HDACs (data not shown). Altogether, these results indicate that on a chromosomally integrated ER-responsive transgene, HDAC inhibition modifies the antiestrogen behaviour.

**Characterization of the TSA-induced agonist activity of OHTam**

To analyze further the effect of HDAC inhibition on ER activity, time-course experiments were...
performed. As shown in Fig. 2B, the TSA-induced (hatched bars) agonist activity of OHTam was detectable as soon as 6 h after treatment beginning. The rapid induction of agonist activity was supported by the analysis at the luciferase mRNA level which demonstrated that the increase upon TSA plus OHTam treatment was insensitive to cycloheximide and therefore did not require the synthesis of an intermediate protein (data not shown). When we analyzed the TSA effect over longer periods of time, we noticed that OHTam agonist activity was maximal (2.9-fold increase over control) after 16 h of treatment in the presence of TSA and slightly decreased after 24 h of incubation (Fig. 2B). In this system, the transrepressive activity of OHTam was also observed after 6 h of treatment in the absence of TSA and was maximal after 24 h (32% of control levels).

We then investigated the effect of TSA on the response to other antiestrogens. As shown in Fig. 2C, partial antiestrogens such as raloxifen or the LY117018 compound clearly exhibited agonist activity in the presence of TSA (hatched bars). By contrast, in the presence of the pure antiestrogen ICI182780, TSA abolished the transrepressive activity but did not induce any significant agonist activity (Fig. 2C). The absence of agonist effect of ICI182780 in TSA-treated cells was obtained for concentrations of antihormone ranging from 0.1 nM to 0.1 µM (data not shown).

As shown in Fig. 3A, dose–response experiments performed with OHTam showed that the half-maximal concentration (EC50) for agonist activity in the presence of TSA (hatched bars) was obtained between 1 nM and 10 nM. In parallel, the relative binding affinity of OHTam for ERβ was determined (about 8 nM) and it did not appear significantly different in the presence or absence of TSA (data not shown). Moreover, the experiment shown in Fig. 3B clearly indicated that the pure antiestrogen ICI182780 totally prevented the

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**Figure 2** Effect of TSA on OHTam activity in stably transfected MCF7 cells. (A) MELN cells were treated for 24 h with the indicated concentrations of TSA or FR901228 in the absence of ligand (white boxes) or in the presence of 10 nM E2 (black boxes) or OHTam (grey boxes). Results are expressed as relative luciferase units (RLU; percentage of values obtained in the presence of E2). (B) MELN cells were treated for the indicated periods of time with 10 nM OHTam in the presence (hatched boxes) or absence (white boxes) of TSA (500 ng/ml). Results are expressed as RLU (% of control). (C) MELN cells were treated for 24 h in the absence or presence of different ER ligands at 10 nM: E2, OHTam, Raloxifen (RAL), LY117018 (LY), ICI182780 (ICI), or with solvent alone (Control) in the presence or absence of TSA (500 ng/ml). Results are expressed as RLU (percentage of values obtained in the presence of E2) and correspond to the means±S.D. of three independent values.
TSA-induced increase of OHTam agonist activity. All these data strongly support an ER-mediated effect.

Transient expression of exogenous ERα in MELN cells

Since ERα expression was markedly repressed by TSA in MCF7 cells (Fig. 1), we investigated the role of ERα levels in the TSA-induced agonist activity of partial antiestrogens. We therefore transiently transfected MELN cells with an ERα expression vector to counterbalance the diminution of endogenous receptor levels. Results shown in Fig. 4C indicated that, in the absence of TSA (left panel), the relative transcriptional activity of ERα in response to E2 and OHTam was not modified when wild-type receptor was overexpressed. In the presence of TSA (right panel), transient transfection of ERα expression vector decreased OHTam agonist behaviour without restoring its inhibitory activity.

Transient transfections were also performed using expression vectors for mutant ERα (Fig. 4A). These mutants are either deleted of the activation domains, AF1 (HE19) or AF2 (HE15), or mutated in helix 12 (AF2 mut) or on lysines K302 and K303 (Kmut) which have been described as p300 acetylation sites (Wang et al. 2001). When assessed by Western blot, all these mutants showed comparable expression levels except the HE15 mutant which gave a higher signal (Fig. 4B). The HE19 and Kmut mutant receptors behaved as the wild-type receptor and decreased the OHTam agonist activity (Fig. 4C). By contrast, the HE15 and AF2 mut mutants did not modify the relative response to E2 and OHTam. Hence, these results indicate that exogenous expression of ERα with a functional AF2 domain is able to reverse the TSA-induced OHTam agonist activity in MELN cells.

Stable expression of ERα or ERβ in MELN cells

To further emphasize the effect of exogenous ER expression, we stably introduced ER (α or β) expression vector into MELN cells to generate MELN-ERα and MELN-ERβ cells. These double transfectants were screened by ER quantification using a ligand-binding assay and, in both cases, independent clones were isolated which all gave similar results concerning ER expression and luciferase regulation (see Fig. 5 for results with representative clones).

The basal expression of ERα in MELN-ERα clones was surprisingly not modified as compared with parental or mock-transfected MELN cells (Fig. 5A). In the presence of TSA, a clear difference in receptor expression was however noticed, ERα levels being strongly reduced in MELN cells transfected with empty pSG5 vector (MELN-mock) and in MELN-ERβ whereas it was slightly increased in the MELN-ERα clones. This resulted from the decrease of endogenous ERα combined with the induction of exogenous ERα which is under the control of the TSA-induced SV40

Figure 3 Dose–response experiment and effect of ICI182780. (A) MELN cells were treated for 16 h with increasing concentrations of OHTam in the absence (white boxes) or presence (hatched boxes) of TSA (500 ng/ml). (B) MELN cells were treated for 16 h in the presence of TSA (500 ng/ml) with different ER ligands: E2, OHTam or ICI182780 (ICI) at 10 nM or by a combination of E2 or OHTam plus ICI at 1 μM. Results are expressed as relative luciferase units (RLUs; percentage of values obtained in control conditions (A) or in the presence of E2 (B)) and correspond to the means±s.d. of three independent values.
promoter. As shown in Fig. 5A, endogenous ERβ expression was not observed either in MELN-mock or in MELN-ERα cells. The ERβ protein was, however, specifically detected in the MELN-ERα transfectants and its expression significantly increased by TSA (due to the SV40 promoter regulation). When measured by ligand-binding assay, the relative expression of ERβ in the absence of TSA was estimated at about 30% that of endogenous ERα.

Concerning the effect of TSA on ER transcriptional activity, the mock-transfected MELN cells responded as the parental cells whereas in MELN-ERα and MELN-ERβ cells, the agonist activity of OHTam upon TSA treatment was completely lost (Fig. 5B). These results thus reinforce our data showing the importance of ERα expression (Fig. 4) and demonstrate that ERβ is also able to compensate for the TSA-dependent decrease of endogenous ERα levels.

Moreover, in both MELN-ERα and MELN-ERβ cells, TSA treatment relieved OHTam inhibitory activity supporting the idea that HDAC activity is required for active transcriptional transrepression by OHTam-ligated ERα and β.

By contrast, we noticed that in MELN-ERα cells, the pure antiestrogen ICI182780 still decreased ERα activity in the presence of TSA (Figure 5B). Interestingly, in the presence of TSA, the repressive properties of ICI182780 were not
observed in MELN-mock or in MELN-ERβ which both presented a marked TSA-dependent decrease of ERα levels (Fig. 5A and B). Taken together these findings provide evidence that in our MCF7 system with a chromosomally integrated transgene, HDAC inhibition strongly regulates the relative agonist/antagonist activity of antiestrogens. Our data indicate that part of these effects, i.e. induction of agonist activity with partial antiestrogens, involves a mechanism linked to the level of endogenous ERα expression.

**Inhibition of ERα levels in MELN cells**

To evaluate precisely the relative role of ERα expression on the transcriptional response to OHTam, we sought to decrease ERα levels in MELN cells independently of HDAC inhibition. As shown in Fig. 6, a noticeable reduction of ERα levels was obtained after transient transfection of a specific antisense oligonucleotide covering the first ATG. This produced no significant variations either in the regulation by E2 in the presence or absence of TSA (data not shown) or in the agonist activity of OHTam in the presence of TSA. By contrast, lowering ERα levels in the absence of TSA resulted in a complete blockade of the transrepression properties of OHTam. These
results support the hypothesis that the relative expression of ERα is an important factor for the control of the relative agonist/antagonist activity of partial antiestrogens.

Discussion

In the present study, we have analyzed the effect of HDAC inhibition on ER transcriptional activity in response to antiestrogens. The main conclusion of this work is that inhibition of HDAC activity combined with low ERα levels switches partial antiestrogens from antagonists to agonists in MCF7 cells with a chromosomally integrated ER-regulated transgene (MELN cells). In this model, TSA treatment induces transcriptional activation by OHTam or raloxifen but not by the pure antiestrogen ICI182780. This regulation of antiestrogens from antagonists to agonists in MCF7 cells blocked by ICI182780; (2) the EC50 corresponds to the OHTam affinity for ERα; and (3) it is not observed in HeLa cells without expression of the exogenous receptor (data not shown). This OHTam agonist activity requires the concomitant TSA-dependent decrease in ERα expression as it is lost when exogenous receptor is expressed upon stable or transient transfection. Interestingly, a functional AF2 domain is required since overexpression of ERβ or AF1-deleted ERα (but not of an AF2-mutated receptor) is sufficient to abolish the agonist activity of OHTam (Figs 4 and 5). The importance of ERα levels has been corroborated in an ER-negative model (HeLa cells stably integrated with the same reporter as in MELN), where a slight agonist activity of OHTam has been obtained in the presence of TSA only when low levels of ERα were expressed (data not shown).

A hypothesis that could explain this effect is based on the acetylation of ERα itself. It has been shown that lysines K302 and K303 are the main acetylation sites for p300 on ERα and the replacement of these two residues by alanines altered receptor activity by increasing ligand sensitivity and decreasing the TSA response (Wang et al. 2001). Acetylation of the receptor could alter the binding affinity of proteins involved in transcriptional regulation and preferentially affect those interacting with the hinge region of nuclear receptors, such as the L7/SPA protein (Jackson et al. 1997), c-jun (Teyssier et al. 2001) or calmodulin (Garcia Pedrero et al. 2002). However, our results do not support this hypothesis since first, Western blot analysis using different antibodies against acetylated lysine did not give any significant signal with immunoprecipitated ERα and second, in our hands, the transcriptional activity of the ERα double-mutant K302A/K303A was identical to that of the wild-type receptor (data not shown).

Another explanation for the TSA-dependent agonist activity of OHTam implicates the regulation by TSA of transcription cofactor levels. Nevertheless, such a regulation needs to be direct in regard to our observation that protein synthesis inhibition by cycloheximide did not modify the response to TSA (data not shown). Some of the nuclear receptor transcription cofactors (N-CoR, p300, BRG-1 or BAF155) are indeed negatively regulated by TSA (Wilson et al. 2002). However, the fact that exogenous ERα expression abolishes OHTam agonist activity in the presence of TSA suggests that it is not the absolute expression of cofactors which is crucial but rather their relative expression as compared with that of the receptor. Alternatively, another hypothesis could involve an increased acetylation of cofactors upon TSA treatment based on the demonstration that their association with either the receptor or downstream effectors could be modified according to their acetylation status, as shown respectively for SRC-3 (Chen et al. 1999) and RIP140 (Vo et al. 2001).

The second conclusion of the present study is that the capacity of partial but not pure antiestrogens to repress ERα activity requires HDAC enzymatic activity. The transrepression of partial antiestrogens (i.e. their ability to decrease ERα activity under the level obtained with unliganded receptor) is abolished by TSA treatment. This effect is independent of the decrease in ERα levels since it is also observed when ERα or β is overexpressed upon stable transfection in MELN cells. It has been recently reported using chromatin immunoprecipitation experiments that OHTam and raloxifen induce the recruitment of HDAC2 and HDAC4 to estrogen target promoters (Shang & Brown 2002), thus indicating that these proteins might play an important role. Our observations support this result and give the demonstration that the enzymatic activity of HDACs is necessary for transcriptional repression by OHTam-liganded receptors. By contrast, previous studies have reported that pure antiestrogens induce a particular
conformation of ER (Pike et al. 2001) which does not allow the in vitro interaction of ERα with the corepressor N-CoR (Metivier et al. 2002). The inhibitory effect of ICI182780 rather implicates a decrease in ERα stability (Wijayaratne & McDonnell 2001) and/or mobility (Stenoien et al. 2001). However, we have found that the inhibitory activity of ICI182780 is lost in MELN cells upon TSA treatment and this is probably related to the fact that TSA treatment by itself produces a strong decrease of ERα accumulation. Interestingly, in MELN-ERα cells, where exogenous ERα is constitutively expressed, the transrepression activity of ICI182780 is maintained even in the presence of HDAC inhibitor thus supporting an HDAC-independent mechanism for the inhibitory effect of ICI182780. On the other hand, in MELN-ERβ, the transrepression behaviour of ICI182780 is not significantly restored upon exogenous ERβ expression. This suggests that ERβ stability might not be affected to the same extent in the presence of pure antiestrogens, in support of our recent observation showing a differential effect of ICI182780 on the accumulation of ERα and ERβ proteins (Margeat et al. 2003).

The third conclusion is that the antagonist activity of OHTam is lost when endogenous ERα levels are decreased, independently of HDAC inhibition, using antisense oligonucleotides. Previous studies have shown that the relative expression of coactivators versus corepressors is critical for the agonist behaviour of partial antihormones (Jackson et al. 1997, Shang & Brown 2002). These data altogether suggest that the transcriptional response to partial antihormones could depend on the balance between ERs and their transcription cofactors. Hence, modulation of either of these parameters (i.e. expression levels of coactivators, corepressors or receptors) could be of importance in determining the relative agonist/antagonist activity of antihormones in breast cancer cells.

From a clinical point of view, our data are consistent with previous studies dealing with the response of patients to endocrine therapy in relation to ER levels. These papers showed that the rate of response to tamoxifen treatment was significantly correlated with ER concentration (Williams et al. 1987, Anderson et al. 1989). Almost all patients who progressed on treatment had tumors with a low (less than 20 fmol/mg cytosol protein), but in most cases detectable, ER concentration (Anderson et al. 1989). In addition, partial agonist effects of tamoxifen have also been observed in various human tissues such as bone and uterus. In endometrial cancer cells, the agonist activity has been reported both at the level of gene expression and cell proliferation (Jamil et al. 1991, Barsalou et al. 1998). This explains why its use in breast cancer hormone therapy has been associated with an increased risk of endometrial carcinoma (Goldstein 2001).

It would be of great importance to define whether the equilibrium between ERα and transcription cofactors, associated or not with differences in the cellular acetylation status, is modified in these physio-pathological situations.

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