Anti-estrogenic actions of histone deacetylase inhibitors in MCF-7 breast cancer cells

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Abstract

Anti-estrogens are the current endocrine therapy of choice in the treatment of estrogen receptor (ER)-positive breast cancers. Histone deacetylase inhibitors (HDACi) also constitute a promising treatment for therapy, and combination of anti-estrogens with HDACi may improve efficacy while reducing side effects. We have examined the effect of the HDACi sodium butyrate and suberoylanilide hydroxamic acid (SAHA), alone and in combination with 17β-estradiol (E2) and the pure anti-estrogen ICI 182.780 (ICI) in human MCF-7 breast cancer cells. HDACi caused a sustained increase of histone H3 acetylation and caused cell death as shown by flow cytometry analysis. In surviving cells, both inhibitors were even stronger than ICI in depleting cyclin D1 levels, inducing expression of the cyclin kinase inhibitor p21Waf1/Cip1, blocking phosphorylation of the retinoblastoma protein, or inhibiting cell growth. No additive effects of ICI with either butyrate or SAHA were found. In addition, these drugs were able to antagonize the effects of E2 on expression of cell cycle proteins, cell growth, and transcription of ER-dependent genes. The anti-estrogenic effects of HDACi appear to be related to a strong downregulation of the expression of ERα that appears to be secondary to both transcriptional and post-transcriptional regulation. ERα phosphorylation is involved in estrogen signaling, and HDACi also prevented receptor phosphorylation in Ser-118 both in the absence and presence of ER ligands. These results provide further support for the use of deacetylase inhibitors as chemotherapeutic agents in the treatment of breast cancer tumors.

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Introduction

Estrogen plays a key role in normal breast development, as well as in growth and progression of breast cancer. The biological actions of estrogen are mediated by binding to nuclear estrogen receptors (ERα and ERβ). In breast cancer cells expressing ERs, estrogen has potent proliferative effects and also affects differentiation and survival. Multiple cell cycle regulatory pathways, including an increase in cyclin D1 expression, p21Waf1/Cip1 redistribution, and modulation of cyclin E–cyclin-dependent kinase 2 (CDK2) activity are regulated by estrogen. These changes lead to hyperphosphorylation of proteins of the retinoblastoma (pRB) family and progression through the cell cycle (Musgrove et al. 1994, Prall et al. 1998, Cariou et al. 2000, Bjornstrom & Sjoberg 2005).


Tamoxifen, a selective ER modulator that has anti-estrogenic effects in the breast, is the current endocrine therapy of choice in the treatment of ERα-positive breast
cancers. However, development of resistance to tamoxifen therapy has led to the development of pure steroidal anti-estrogens such as ICI 182.780 (ICI), potentially more effective for breast cancer treatment (Howell et al. 1995). A key event for the anti-proliferative effects of anti-estrogens appears to be the downregulation of cyclin D1 (Musgrove et al. 1993, Cicatiello et al. 2004). The p21\textsuperscript{Waf1/Cip1} that is released from cyclin D1–CDK4 complexes after the anti-estrogen-induced decrease in cyclin D1 can then bind cyclin E–CDK2 complexes, inhibiting its enzymatic activity and causing growth arrest (Carroll et al. 2000).

Combination therapy involving anti-estrogens and other cytotoxic drugs may improve efficacy for the treatment of breast cancer. Among these drugs, histone deacetylase inhibitors (HDACi) constitute a promising treatment due to their low toxicity, and HDACi are currently being tested in clinical trials (Marks et al. 2004, Minucci & Pelicci 2006). HDACi have been shown to induce G\textsubscript{1}-phase cell cycle arrest with downregulation of cyclin D1 and upregulation of p21\textsuperscript{Waf1/Cip1} in breast cancer cells (Chopin et al. 2002, Alao et al. 2004, Margueron et al. 2004). It is becoming clear that ER and HDACi pathways crosstalk at various levels such as expression and activity of ER\textsubscript{z}, regulation of p21\textsuperscript{Waf1/Cip1} expression, cell proliferation, etc. (Reid et al. 2005).

In this study, we have analyzed the effect of the short-chain fatty acid sodium butyrate and of suberoylanilide hydroxamic acid (SAHA), a second generation HDACi that induces differentiation of breast cancer cells (Munster et al. 2001) on MCF-7 cell proliferation in combination with 17\textbeta-estradiol (E\textsubscript{2}) and ICI 182.780 (ICI). Our results show that HDACi were even more potent than ICI to regulate expression of cell cycle proteins and cell growth. These changes are accompanied by a marked depletion of ER\textsubscript{z} expression that appears to be secondary to both transcriptional and post-transcriptional regulation, and of ER\textsubscript{z} phosphorylation in Ser-118. As a consequence, HDACi were able to antagonize E\textsubscript{2}-dependent responses reinforcing the idea that these drugs could be useful in the treatment of breast cancer. In contrast, no cooperative effects of HDACi with ICI on expression of cell cycle proteins or inhibition of ER signaling were observed, suggesting that this combination might not result in improved efficacy.

**Materials and methods**

**Cell proliferation**

MCF-7 cells were grown in DMEM–HEPES containing 10% fetal calf serum. Cells were inoculated in six-well plates at 35–40×10\textsuperscript{3} cells/well and, 24 h before the beginning of treatments, cells were shifted to medium containing AGX100 resin–charcoal-treated serum to eliminate steroid hormones. Cells were treated with 2 mM sodium butyrate, 1 \textmu M SAHA, 100 nM E\textsubscript{2}, or 100 nM ICI 182.780 (ICI). Cells were counted in Neubauer chambers. For determination of total cell protein, cultures were washed with PBS and lysed as previously described (Perez-Juste & Aranda 1999). Total protein was determined with the BCA protein assay (Pierce, Rockford, IL, USA).

**Flow cytometry**

Triplicate cultures of MCF-7 cells grown in 60 mm Petri dishes were transferred to the medium containing depleted serum and after 24 h incubated with butyrate or SAHA for 48 h. Both floating and adherent cells were collected, washed twice with cold PBS, fixed with chilled ethanol 70%, and centrifuged. Pellets were incubated RNase A and stained with propidium iodide for sorting as previously described (De los Santos et al. 2007). The percentage of cells in sub-G\textsubscript{1}-, G\textsubscript{1}-, S-, and G\textsubscript{2}/M-phases was calculated with WinMDI and Cylchred software for Windows.

**Western blot**

Proteins from cell lysates were separated in SDS-PAGE and transferred to PDVF membranes (Immobilon, Millipore, Bedford, MA, USA) that were blocked for 1 h at room temperature with 4% BSA. Incubation with primary antibodies (Garcia-Silva & Aranda 2004, De los Santos et al. 2007) was performed overnight at 4 °C, and with the secondary antibody for 1 h at room temperature. Blots were visualized with ECL (Amersham). Antibodies against cyclin D1, p21\textsuperscript{Waf1/Cip1}, and hyperphosphorylated pRb were obtained from Santa Cruz, Inc, Santa Cruz, CA, USA. The ER\textsubscript{z} antibody was a kind gift from S Ramos, and the antibody against ER\textsubscript{z} phosphorylated in Ser-118 was obtained from Santa Cruz. These antibodies were used at a 1:2000 dilution. The antibody for acetylated histone H3 in lysine 9 and 14 (Upstate, Charlotteville, VA, USA) was used at a 1:5000 dilution. Anti-human actin (Santa Cruz) antibody was used as a loading control.

**Real-time PCR**

Total RNA was extracted using Tri-Reagent (Sigma), and mRNA levels were analyzed by quantitative real-time PCR (Q-RT-PCR). RT was performed with 2 \mu\text{g} RNA following specifications of SuperScript First-Strand Synthesis System (Invitrogen Life Technologies). PCRs were performed in a Rotor Gene
and detected with SYBR Green using the following primers: ERα 5'-CCACCAACCAGTGCACATT-3' (forward) and 5'-GGTCTTTTGATCCCACCTTT-3' (reverse); PR 5'-ATCAACTAGCGAGGACACCT-3' (forward) and 5'-TGCAAAACCTGGCAATGATT-3' (reverse); pS2 5'-TCCCTGTGGCTCTATCCCAA-3' (forward) and 5'-AGTGTC-TAAAATTCACACTCCTGT-3' (reverse). Results were analyzed by the C_T comparative method (ΔΔC_T).

Results

The anti-proliferative effect of sodium butyrate (2 mM) and SAHA (1 μM) in human MCF-7 breast cancer cells is shown in Fig. 1A. Incubation with the HDACi reduced cell counting after 48 h, and this effect was more apparent after 4 days, where the number of cells was reduced by about 50 and 70% in cells treated with 1 μM SAHA and 2 mM butyrate respectively. Flow cytometry analysis demonstrated that these compounds produced MCF-7 cell death, manifested by accumulation of sub-G1 cell debris. A significant percentage of cells treated with butyrate or SAHA were in sub-G1 after 48 h of incubation (Fig. 1B).

Proliferation of breast cancer cells is tightly linked to expression of cyclin D1 and the CDK inhibitor (CKI) p21WAF1/Cip1. A major function of cyclin D/CDK complexes is the phosphorylation of the tumor suppressor pRb that is required for the progression through the cell cycle. Therefore, the levels of these proteins were determined by western blot in MCF-7 cells treated with the HDACi alone and in combination with E2 and the pure antagonist ICI. At 24 h of treatment with the different compounds, obvious differences in the levels of hyperphosphorylated pRb were not found (Fig. 2A). In contrast, after 48 h, strong changes in pRb phosphorylation were observed (Fig. 2B). Whereas E2 caused a marked increase in phosphorylation, SAHA, butyrate, and the anti-estrogen caused an important reduction in the levels of P-pRb. Furthermore, both HDACi were able to block E2-dependent pRb hyperphosphorylation, and in cells treated with E2 in combination with the HDACi the levels of P-pRb were even lower than in control cells. In contrast, a further reduction was not found when the HDACi were combined with ICI.

Cyclin D1 expression closely paralleled pRb phosphorylation after the different treatments. E2 caused the expected increase in cyclin D that was more noticeable after 48 h, whereas ICI had a converse effect. Butyrate was slightly stronger than SAHA to repress cyclin D1 expression, and the reduction in the levels of this protein caused by both HDACi was even more pronounced than that caused by ICI. As in the case of pRb phosphorylation, butyrate and SAHA counteracted the stimulatory effect of E2 on cyclin D1 expression, although again the effect of butyrate was slightly stronger.

The levels of the CKI p21WAF1/Cip1 correlated inversely with those of phosphorylated pRb and cyclin
D1 in HDACi-treated cells. Both butyrate and SAHA caused a sustained increase in p21\text{Waf1/Cip1} expression that was observed both in the absence and presence of the steroid and the pure anti-estrogen that by themselves did not appreciably alter the levels of this CKI.

On the other hand, butyrate and SAHA produced a sustained increase in histone H3 acetylation that was detectable even after 48 h. Interestingly, both HDACi were equally potent to induce acetylation, although regulation by butyrate of P-pRb, cyclin D1, and p21\text{Waf1/Cip1} levels was somewhat more accentuated.

The ability of HDACi to antagonize the effect of E\textsubscript{2} on expression of proteins important for cell cycle progression suggested that these compounds could also suppress estrogen-dependent MCF-7 cell growth. As shown in Fig. 3, HDACi reduced cell number (Fig. 3A) as well as the total amount of cellular protein per culture (Fig. 3B), and was able to block E\textsubscript{2}-dependent increase in cell proliferation. Also, in parallel with the lack of cooperation of HDACi and ICI on regulation of cell cycle proteins, a further reduction in neither cell number nor total protein content was observed when cells were treated with either butyrate or SAHA in combination with the estrogen antagonist.

ER\textsubscript{a} expression in breast cancer cells has been found to be reduced by other HDACi (Reid \textit{et al.} 2003, Alao \textit{et al.} 2004). We then analyzed by western blot the effect of 48-h incubation with butyrate (Fig. 4A) and SAHA (Fig. 4B) alone and in combination with E\textsubscript{2} and ICI on ER\textsubscript{a} levels in MCF-7 cells. Incubation with the different compounds caused downregulation of receptor expression, and this reduction was maximal when HDACi and ER ligands were administered together. Post-translational modifications of ER\textsubscript{a} are emerging as important regulatory elements of crosstalk between different signaling pathways. In particular, phosphorylation at Ser-118 has been implicated in the ligand-dependent and ligand-independent effects of ER\textsubscript{a} and in tamoxifen resistance of breast tumors (Lonard \textit{et al.} 2000, Wijayaratne & McDonnell 2001, Murphy \textit{et al.} 2004). As shown in Fig. 4, incubation of MCF-7 cells with either butyrate or SAHA caused a total depletion of ER\textsubscript{a} phosphorylation in Ser-118 (P-ER\textsubscript{a}) in parallel with the downregulation of receptor levels. This was different from that observed with E\textsubscript{2} and ICI that also reduced total ER\textsubscript{a} levels but did not deplete P-ER\textsubscript{a}. In addition, receptor phosphorylation was undetectable when the ER ligands were combined with either butyrate or SAHA.

ER\textsubscript{a} is degraded through the ubiquitin–proteasome pathway (Lonard \textit{et al.} 2000, Wijayaratne & McDonnell 2001), and it has been shown that MG132, a proteasome inhibitor, relieves the decrease of ER\textsubscript{a} mediated by the HDACi trichostatin A (TSA) and valproate (VPA; Reid \textit{et al.} 2003). We then analyzed the influence of MG132 treatment on ER\textsubscript{a} levels in MCF-7 cells treated with butyrate alone and in combination with E\textsubscript{2} and ICI for 24 h. As shown in Fig. 5A, in the absence of the inhibitor, this time period was sufficient to cause a marked reduction of ER\textsubscript{a} expression by the different compounds, and again this reduction was maximal when the ER ligands were combined with the HDACi. Blockade of proteasome activity with MG132 partially prevents receptor downregulation by butyrate, E\textsubscript{2}, or ICI alone, but was unable to restore ER\textsubscript{a} levels when these agents were combined suggesting that, in addition to an increase in receptor turnover, changes in gene expression could underlie the decrease in ER\textsubscript{a}. Indeed, as shown in

Figure 3 HDACi inhibits estradiol-dependent MCF-7 cell growth. Cells were treated with medium alone, 100 nM E\textsubscript{2} or 100 nM ICI 182,780 in the presence and absence of 2 mM butyrate (But) and 1 \textmu M SAHA. Cells were counted (A) and total protein content of the cultures (B) was determined after 48 h incubation with these compounds.

Figure 4 HDACi downregulates ER\textsubscript{a} expression. (A) MCF-7 cells were treated for 48 h with 17\textbeta-estradiol (E\textsubscript{2}) and ICI 182,780 (ICI) alone or in combination with sodium butyrate (But) (A) or SAHA (B) as indicated. In these cells as well as in control cells (C), the levels of total ER\textsubscript{a}, ER\textsubscript{a} phosphorylated in Ser-118 (P-ER\textsubscript{a}), and actin were determined by western blot. Arrows indicate the position of the specific bands.
Fig. 5B, neither E2 nor ICI reduced the steady-state level of ERα mRNA in MCF-7 cells. In contrast, butyrate reduced ERα transcripts by more than 80% independently of the presence of the ER ligands.

To determine the effect of ERα depletion by butyrate on E2-dependent gene expression, we measured transcripts for the well-known estrogen target genes progesterone receptor (PR) and pS2 that have EREs in their regulatory regions. As shown in Fig. 6, butyrate had an effect similar to that of ICI on basal PR and pS2 transcripts, since these compounds did not alter PR mRNA but appreciably reduced the levels of pS2 mRNA. In addition, the anti-estrogenic effects of butyrate were as strong as those of ICI, and the HDACi was able to block the transcriptional response of both genes to E2.

Discussion

We have analyzed the effect of the HDACi sodium butyrate and the hydroxamic acid derivative SAHA on growth of estrogen-dependent human breast cancer cells. SAHA is the first HDACi approved by the FDA to enter the clinical oncology market and has been successfully used in phase II trials for the treatment of cutaneous T cell lymphoma (Duvic et al. 2007). Our results show that both compounds produced sustained histone hyperacetylation and also caused strong growth inhibition. Inhibition is associated with an increase in apoptotic cell death, as indicated by the appearance of cells in sub-G1. Besides inducing cell death, butyrate and SAHA also caused a significant decrease in the number of surviving cells that progress through the cell cycle. HDACi appears to induce growth arrest through changes in the expression of key regulatory components of cell cycle progression such as cyclin D1 or the CKI p21Waf1/Cip1, previously described to be regulated by HDACi in breast cancer cells (Huang et al. 2000, Chopin et al. 2002, Alao et al. 2004). Cyclin D1 is frequently overexpressed in human breast cancers (Gillett et al. 1994) and has been implicated in the development of mammary hyperplasia and carcinogenesis (Wang et al. 1994). Furthermore, cyclin D1 expression can enable cells arrested by growth factor deprivation and treatment with anti-estrogens to complete the cell cycle (Musgrove et al. 1994). We have confirmed that estrogen induces cyclin D1
expression, whereas anti-estrogens have an inhibitory effect, and we have additionally observed that HDACi can counteract induction of cyclin D1 expression as well as E2-dependent growth. Interestingly, repression of cyclin D1 expression by butyrate or SAHA was stronger than that found with the pure anti-estrogen ICI, and this compound did not further reduce HDACi-mediated cyclin D1 expression.

The Waf/Kip family of CKIs that includes among other p21Waf1/Cip1 inhibits kinase activity of G1/S CDKs (Sherr & Roberts 1999). In agreement with previous observations (Huang et al. 2000, Chopin et al. 2002, Margueron et al. 2004), HDACi caused an important increase of p21Waf1/Cip1 expression in MCF-7 cells. However, at difference with other reports (Cariou et al. 2000, Varshochi et al. 2005), with the culture conditions used and at the times analyzed, we could not observe significant changes in p21Waf1/Cip1 levels by either E2 or ICI. Furthermore, the ER ligands did not alter p21Waf1/Cip1 induction by HDACi.

A major function of the complexes of CDKs with G1-specific cyclins is the phosphorylation of pocket proteins, such as the tumor suppressor pRb. Hyper-phosphorylation of pocket proteins releases E2F transcription factors, which can then activate expression of genes required for progression through the S-phase (Sherr & Roberts 1999). Correlating with the reduction in cyclin D1 and the increase in expression of the CKI, treatment with HDACi induced the same effect as ICI treatment, leading to a profound downregulation of pRb phosphorylation that should eventually arrest MCF-7 cell growth. As in the case of cyclin D1 downregulation or p21Waf1/Cip1 induction, the reduction in pRb phosphorylation was already maximal in cells incubated with HDACi alone as was not further decreased when these drugs were combined with ICI. The lack of cooperative effects of HDACi and the pure anti-estrogen on expression of cell cycle proteins can explain why these drugs did not cooperate to induce MCF-7 growth arrest. Also in agreement with the antagonism on cyclin D1 induction, HDACi were able to block E2-dependent pRb phosphorylation, demonstrating again the strong anti-proliferative effects of these inhibitors in estrogen-dependent breast cancer cells. In relation to this, it has been shown that ER-positive breast cancer cells are more sensitive to the HDACi TSA than ER-negative cells (Reid et al. 2003, Alao et al. 2004, Margueron et al. 2004).

The anti-estrogenic effects of HDACi could be related to the depletion of ERα levels previously observed in breast cancer cells (Reid et al. 2003, Alao et al. 2004). ERα is degraded through the ubiquitin–proteasome pathway in response to both estrogen and ICI binding (Lonard et al. 2000, Wijayaratne & McDonnell 2001), demonstrating that ligand-dependent receptor degradation does not depend on transcription. Our results show that ERα depletion is maximal with the combination of HDACi and E2 or ICI. Furthermore, MG132 cannot relieve the decrease of ERα accumulation under these conditions, suggesting that this effect could be at least in part secondary to transcriptional regulation. This was indeed confirmed by measuring ERα transcripts that were found strongly reduced in cells incubated with HDACi. Our results reinforce previous observations (Alao et al. 2004), and additionally show that this regulation occurs independently of ERα occupancy since neither E2 nor ICI further increased butyrate-mediated ERα mRNA downregulation.

Ser-118 is a well-studied phosphorylation site in ERα. Both estrogens and growth factors (Kato et al. 1995, Bunone et al. 1996, Chen et al. 2000, 2002) can result in Ser-118 phosphorylation and, recently, it has been shown that ICI can also trigger this receptor modification (Lipfert et al. 2006). Our data show that ICI is at least as strong as E2 to induce a sustained increase of Ser-118 ERα phosphorylation in MCF-7 cells. Although after 48-h treatment a net increase in the levels of the modified protein was not observed in ICI-treated cells, ER occupancy reduced very significantly total ERα levels, and therefore the ratio of unmodified versus phosphorylated receptor increases very significantly. Our data also provide evidence that upon HDACi treatment ERα phosphorylation becomes undetectable in MCF-7 cells and that this occurs even in the presence of ER ligands. Interestingly, it has been proposed that phosphorylation in Ser-118 may be associated with increase in E2 agonism, progression of breast cancer, resistance to tamoxifen therapy, and estrogen-independent growth of MCF-7 cells (Likhite et al. 2006, Murphy et al. 2006). Clearence of phosphorylated receptor could also contribute to the E2-independent and E2-dependent growth arrest secondary to HDACi treatment observed in this study.

Transcriptional silencing of estrogen target genes in response to deacetylase inhibition by VPA and TSA has been reported (Reid et al. 2005), and consistent with the finding that butyrate and SAHA drastically reduced total and phosphorylated ER levels, we have demonstrated that they also abolish E2-dependent transcription of the ER target genes, PR and pS2. Progesterone plays an important role in mammary gland physiopathology, and PR as well as pS2 has been used as an indicator of breast cancer progression and a predictor for tamoxifen resistance of breast tumors (Johnston et al. 1995). On the other hand, the effect of butyrate on gene expression
parallels closely that of ICI, showing again the anti-
estrogenic actions of HDACi. As also observed with the
depletion of cyclin D1 and pRB phosphorylation or with
the induction of p21Waf1/Cip1 levels, the effects of HDACi
alone were already maximal and were not further
enhanced by the antagonist.
In conclusion, ours results show that butyrate and
SAHA appear to have stronger effects than the pure
steroidal anti-estrogen ICI on expression of cell cycle
proteins, downregulation of ER levels, and transcription
of ER target genes in breast cancer cells. The observed
effects provide further support for the use of
deaetylase inhibitors as chemotherapeutic agents in
the treatment of both estrogen-dependent and estrogen-
independent breast cancer tumors.

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References

P, Varshochi R, Stavropoulou AV, Coombes RC &
Vigushin DM 2004 Histone deacetylase inhibitor
trichostatin A represses estrogen receptor alpha-depen-
dent transcription and promotes proteasomal degradation
of cyclin D1 in human breast carcinoma cell lines.
Clinical Cancer Research 10 8094–8104.
Aranda A & Pascual A 2001 Nuclear hormone receptors and
gene expression. Physiological Reviews 81 1269–1304.
Bjornstrom L & Sjoberg M 2005 Mechanisms of
estrogen receptor signaling: convergence of genomic and
nongenomic actions on target genes. Molecular
Endocrinology 19 833–842.
Bunone G, Briand PA, Miksicek RJ & Picard D 1996
Activation of the unliganded estrogen receptor by EGF
involves the MAP kinase pathway and direct phospho-
ylation. EMBO Journal 15 2174–2183.
Butt AJ, McNeil CM, Musgrove EA & Sutherland RL 2005
Downstream targets of growth factor and oestrogen
signalling and endocrine resistance: the potential roles of
c-Myc, cyclin D1 and cyclin E. Endocrine-Related
Cancer 12 S47–S59.
Cariou S, Donovan JC, Flanagan WM, Milic A, Bhattacharya N
& Slingerland JM 2000 Down-regulation of p21Waf1/Cip1 or
p27Kip1 abrogates antiestrogen-mediated cell cycle arrest in
human breast cancer cells. PNAS 97 9042–9046.
Carroll JS, Prall OW, Musgrove EA & Sutherland RL 2000
A pure estrogen antagonist inhibits cyclin E-Cdk2 activity
in MCF-7 breast cancer cells and induces accumulation of
p130-E2F complexes characteristic of quiescence.
Journal of Biological Chemistry 275 38221–38229.
Chen D, Riedl T, Washbrook E, Pace PE, Coombes RC, Egly JM
& Ali S 2000 Activation of estrogen receptor alpha by S118
phosphorylation involves a ligand-dependent interaction
with TFIIB and participation of CDK7. Molecular Cell 6
127–137.
Chen D, Washbrook E, Sarwar N, Bates GJ, Pace PE,
Thirunuvakkarasu V, Taylor J, Epstein RJ, Fuller-Pace FV,
Egly JM et al. 2002 Phosphorylation of human estrogen
receptor alpha at serine 118 by two distinct signal
transduction pathways revealed by phosphorylation-
specific antisera. Oncogene 21 4921–4931.
Chopin V, Toillon RA, Jouy N & Le Bourhis X 2002 Sodium
butyrate induces P53-independent, Fas-mediated apopto-
sis in MCF-7 human breast cancer cells. British Journal of
Pharmacology 135 79–86.
Cicatiello L, Addeo R, Sasso A, Altucci L, Petrizzi VB, Borgo R,
Cancemi M, Caporali S, Caristi S, Scafoglio C et al. 2004
Estrogens and progesterone promote persistent CCND1 gene
activation during G1 by inducing transcriptional derepres-
sion via c-Jun/c-Fos/estrogen receptor (progesterone
receptor) complex assembly to a distal regulatory element
and recruitment of cyclin D1 to its own gene promoter.
Molecular and Cellular Biology 24 7260–7274.
De los Santos M, Zambrano A & Aranda A 2007 Combined
effects of retinoic acid and histone deacetylase inhibitors
on human neuroblastoma SH-SY5Y cells. Molecular
Cancer Therapeutics 6 1425–1432.
Duvic M, Talpur R, Ni X, Zhang C, Hazarika P, Kelly C,
Chiao JH, Reilly JF, Ricker JL, Richon VM et al. 2007
Phase 2 trial of oral vorinostat (suberoylanilide hydro-
xamic acid, SAHA) for refractory cutaneous T-cell
Garcia-Silva S & Aranda A 2004 The thyroid hormone
receptor is a suppressor of ras-mediated transcription,
proliferation, and transformation. Molecular and Cellular
Biology 24 7514–7523.
Gillett C, Fantl V, Smith R, Fisher C, Bartek J, Dickson C,
Barnes D & Peters G 1994 Amplification and overexpression of
cyclin D1 in breast cancer detected by immunohisto-
Howell A, DeFriend D, Robertson J, Blamey R & Walton P
1995 Response to a specific antioestrogen (ICI 182.780)
Huang L, Sowa Y, Sakai T & Pardee AB 2000 Activation of the
p21Waf1 promoter independent of p53 by the histone
daecetylase inhibitor suberoylanilide hydroxamic acid
(SAHA) through the Sp1 sites. Oncogene 19 5712–5719.
Johnston SR, Saccani-Jotti G, Smith IE, Salter J, Newby J,
Coppen M, Ebbs SR & Dowsett M 1995 Changes in
estrogen receptor, progesterone receptor, and pS2
expression in tamoxifen-resistant human breast cancer.
Cancer Research 55 3331–3338.


Musgrove EA, Lee CS, Buckley MF & Sutherland RL 1994 Cyclin D1 induction in breast cancer cells shortens G1 and is sufficient for cells arrested in G1 to complete the cell cycle. PNAS 91 8022–8026.


Prall OW, Rogan EM, Musgrove EA, Watts CK & Sutherland RL 1998 c-Myc or cyclin D1 mimics estrogen effects on cyclin E-Cdk2 activation and cell cycle reentry. Molecular and Cellular Biology 18 4499–4508.


