Functional Activation of the Estrogen Receptor-α and Aromatase by the HDAC Inhibitor Entinostat Sensitizes ER-Negative Tumors to Letrozole

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Abstract

Approximately 25% of breast cancers do not express the estrogen receptor-α (ERα) and consequently do not respond to endocrine therapy. In these tumors, ERα repression is often due to epigenetic modifications such as methylation and histone deacetylation. For this reason, we investigated the ability of the histone deacetylase inhibitor entinostat (ENT) to trigger reexpression of ERα and aromatase in breast cancer cells, with the notion that this treatment would restore sensitivity to the aromatase inhibitor (AI) letrozole. ENT treatment of tumor cells increased expression of ERα and aromatase, along with the enzymatic activity of aromatase, in a dose-dependent manner both in vitro and in vivo. Notably, ERα and aromatase upregulation resulted in sensitization of breast cancer cells to estrogen and letrozole. Tumor growth rate was significantly lower in tumor xenografts following treatment with ENT alone and in combination with letrozole than in control tumors (P < 0.001). ENT plus letrozole also prevented lung colonization and growth of tumor cells, with a significant reduction (P < 0.03) in both visible and microscopic foci. Our results show that ENT treatment can be used to restore the letrozole responsiveness of ER-negative tumors. More generally, they provide a strong rationale for immediate clinical evaluation of combinations of histone deacetylase and aromatase inhibitors to treat ER-negative and endocrine-resistant breast cancers. Cancer Res. 71(5): 1893–903. ©2011 AACR.

Introduction

The knowledge that estrogens play a critical role in growth of hormone-dependent breast cancer is exploited in the use of endocrine therapy such as antiestrogens (AE) and aromatase inhibitors (AI). The development of AIs has led to significant improvements in the treatment of hormone receptor-positive breast cancer in postmenopausal women (1, 2). However, endocrine therapy is limited to patients whose tumors express hormone receptors, namely, the estrogen receptor-α (ERα) and/or the progesterone receptor (PgR). About 25% of breast cancers lack the expression of both hormone receptors and are considered hormone-independent. Systemic therapy for these patients has traditionally been limited to only cytotoxic chemotherapy. Thus, there is a critical need to improve treatment of these women.

Previous reports have suggested that lack of ERα expression in ER-negative breast cancers is due to epigenetic changes, such as increased deacetylation and methylation (3). Histone acetylation and deacetylation are key epigenetic processes that affect gene regulation by changing the DNA conformation in the chromatin. Histone deacetylase (HDAC) is an enzyme that regulates acetylation of histone protein. Recent studies indicate that silencing of a gene by methylation involves the generation of an inactive chromatin structure characterized by deacetylated histones (4). For example, deacetylation of histones results in chromatin condensation, which, in turn, causes transcriptional repression of gene expression (4). CpG island of the ERα gene is extensively methylated in ER-negative breast cancer cells (5) and in about 50% of the primary ER-negative breast tumors but remains unmethylated in normal breast tissue and many ERα-positive tumors and cell lines (5). The relevance of this finding was showed when ER-negative human breast cancer cells were treated with demethylating agent 5-azacytidine. This led to reactivation of ERα mRNA and functional ERα protein (6). Studies have shown that repression of ERα can also be reversed by the use of HDAC inhibitors (HDACis; ref. 6). HDACis such as butyric acid (BA) or trichostatin A reverse the repression by specific inhibition of HDAC activity, leading to histone hyperacetylation, chromatin relaxation, and enhanced transcription. However, this strategy remains to be tested in preclinical in vivo models of breast cancer.
In this study, we tested the hypothesis that inhibiting HDAC in ER-negative tumors will increase expression of functional ERα and aromatase. Thus, tumor cells with silenced ERα expression would be rendered responsive to growth inhibition by AIs. To test our premise, we used the HDAC inhibitor entinostat (ENT/ENT-275/SNDX-275), an oral class I selective HDACi, and the AI letrozole. Our results showed that treatment with ENT resulted in upregulation of ERα and aromatase expression, with consequent inhibition of growth by letrozole of tumor cells in vitro and in vivo. This strategy of converting ER-negative tumors to ER-positive tumors, using HDACis, could provide a new avenue for management of patients with ER-negative breast cancer.

Materials and Methods

Cell culture
ERα-negative MDA-MB-231, Hs578T, and SKBr3 cells were obtained from American Type Culture Collection (ATCC). These cell lines were authenticated by ATCC, using short tandem repeat profiling, karyotyping, and by monitoring cell morphology. In vitro assay conditions and data analysis are described in the Supplementary Methods section.

Subcutaneous tumor analysis
All animal studies were conducted according to the guidelines and approval of the Animal Care Committee of the University of Maryland, Baltimore. Tumor xenografts of MDA-MB-231 cells inoculated into each flank of the female ovariectomized (OVX), athymic nude mouse as previously described with minor modifications (7–12). Treatment details are provided in the Supplementary Methods section. Tumor volumes were calculated from the formula \( V = \frac{4}{3} \pi r_1^2 r_2 \), where \( r_1 < r_2 \).

Lung colonization assay
Mice received injections of \( 3 \times 10^6 \) MDA-MB-231 cells via the tail vein. Groups of mice were treated 3 weeks later with vehicle (control), ENT, letrozole, or ENT plus letrozole. Mice were treated for 6 weeks and then euthanized.

Western blotting
Cell lysates were prepared as described previously (7–11, 13) and 50 µg of protein from each sample was analyzed by SDS-PAGE. The densitometric values were corrected using β-actin as a loading control. Details of antibodies used are described in the Supplementary Methods section.

Aromatase activity
Aromatase activity in cells was determined using a radiometric assay by measuring \( ^{3}H \)H2O formed on conversion of [1-\( ^{3}H \)]androsten3,17-dione (Δ^A; aromatase substrate) to estrone (7, 14).

RNA extraction and reverse transcriptase PCR
RNA was extracted and purified using the RNeasy Mini Kit (Qiagen) as per manufacturer’s protocol. The analysis of ERα, CYP-19 (aromatase), and pS2 mRNA expression was carried out by conventional reverse transcriptase PCR (RT-PCR) as described earlier (8, 15).

Immunohistochemistry
Immunohistochemistry (IHC) was done on formalin-fixed, paraffin-embedded tissue (5-µm sections). The primary antibodies used were ERα and Ki67.

Statistics
For in vitro studies, statistical analysis was done on InStat 3.0 for Mac (GraphPad Software Inc.) One-way ANOVA was used for multiple comparisons with the Tukey test. However, if the data did not pass the normality test, then a nonparametric test was used such as Kruskal–Wallis analysis with Dunn posttest. For in vivo studies, mixed-effects models were used. The tumor volumes were analyzed with S-PLUS (version 7.0; Insightful Corp.) to estimate and compare an exponential parameter (β) controlling the growth rate for each treatment groups. The original values for tumor volumes were log transformed. All P < 0.05 values were considered statistically significant. The graphs are represented as mean ± SEM.

Results

The HDACi ENT induces ERα and aromatase expression in ER-negative breast cancer cells in vitro
Expression of ERα protein was undetectable in MDA-MB-231 cells by Western blotting (Supplementary Fig. 1) and no significant binding of 17β-estradiol (E2) occurred without ENT treatment (data not shown). In addition, growth of MDA-MB-231 cells was not inhibited by AEs or AIs, nor stimulated by estrogen (data not shown). Expression of ERα protein was upregulated 8- and 9.9-fold after treatment with 10 nmol/L of ENT and SAHA, respectively, and expression of aromatase was upregulated 2.6- and 1.8-fold with ENT and SAHA, respectively (Supplementary Fig. 1). Treatment with ENT resulted in upregulation of ERα and aromatase mRNA in a time-dependent manner; an increase was observed in as early as 15 minutes (Fig. 1A). The effect of the 2 HDACis was similar in other ER-negative cell lines, SKBr3 and Hs578T (data not shown). However, ENT was more effective in induction of both ERα and aromatase and was therefore selected for further study.

To show that ERα was functional, a competitive binding study was conducted to quantitate the ERα in MDA-MB-231 cells with or without pretreatment with ENT. In the absence of ENT treatment, E2 did not bind to the ER. When pretreated with 1 µmol/L of ENT for 24 hours (Supplementary Fig. 2), receptor sites occupied by E2 were 1,123 females/mg of protein (βmax).

Next, we evaluated the effect of ENT on estrogen signaling. ENT treatment alone did not change the mRNA levels of pS2, an ERα-induced gene. However, when ENT was followed by treatment with estrogen or Δ^A for 3 hours, pS2 and PgR expression was significantly enhanced (Fig. 1B). These results indicated that while functional ERα protein is expressed with ENT treatment, activation by estrogen is necessary for gene transcription. Furthermore, enhanced transcription of pS2 could be inhibited by letrozole (Fig. 1B).
Figure 1. A, time course of ENT effect on mRNA expression of ER\textsubscript{a} and aromatase: expression of mRNA was examined in MDA-MB-231 cells by RT-PCR at different time points (0–72 hours). Image shows ER\textsubscript{a}, aromatase (CYP-19), and 18s ribosomal RNA (rRNA) as the loading control. B, effect of ENT in the presence or absence of estrogen or \(\Delta^4\)A and letrozole on the mRNA expression of pS2, PgR, and aromatase in MDA-MB-231 cells: RT-PCR analysis shows pS2, PgR, and aromatase (CYP-19) with 18s ribosomal RNA (rRNA) as the loading control. C, effect of ENT on aromatase activity: MDA-MB-231 cells were treated with ENT with or without fulvestrant for 18 hours and then assayed for aromatase activity with (ENT letrozole) or without letrozole (*, \(P = 0.008\) vs. control). D, effect of combining letrozole with ENT or SAHA in MDA-MB-231 cells. Cell viability was measured by the MTT assay after 6-day treatment with increasing concentrations of letrozole alone (IC\textsubscript{50} > 10 \text{nmol/L}) or in the presence of ENT (100 nmol/L). IC\textsubscript{50} value for letrozole was 6.17 \text{nmol/L} when combined with ENT (100 nmol/L). E, effect of \(\Delta^4\)A on response of MDA-MB-231 cells to ENT. The MTT assay was done after 6-day treatment with ENT alone or in the presence of aromatizable \(\Delta^4\)A (10 \text{nmol/L}). F, response of ER-positive or ER-negative cells to \(\Delta^4\)A with or without ENT pretreatment. MDA-MB-231 cells were pretreated with ENT (1 nmol/L) or vehicle for 3 days and then with \(\Delta^4\)A for 6 days. Cell viability was measured after 6 days, using the MTT assay. Ful, fulvestrant; Let, letrozole.
The basal level of aromatase activity in MDA-MB-231 cells was 5.83 ± 1.1 fmol/mg protein/h. When cells were treated with ENT (1 μmol/L) for 24 hours and then incubated with [1β-3H]Δ4Δ for 18 hours, aromatase activity was increased to 87 ± 4.4 fmol/mg protein/h (Fig. 1C). This increase in aromatase activity was dose dependent (data not shown) and showed an increase in functional enzyme. Treatment of MDA-MB-231 cells with SAHA (1 μmol/L for 18 hours) also increased aromatase activity (data not shown), which could then be inhibited by letrozole (1 μmol/L). This increase in aromatase was not dependent on ERα. When cells were pretreated with the ERα downregulator fulvestrant along with ENT, the increase in the aromatase caused by ENT was not affected (Fig. 1C). Thus, these results clearly provided evidence of reexpression of both functional ERα and aromatase following ENT treatment.

As expected, the proliferation of ER-negative MDA-MB-231 cells was not affected by letrozole treatment alone. However, when combined with ENT or SAHA (100 nmol/L), letrozole inhibited cell growth in a dose-dependent manner (IC50 = 6.17 nmol/L; Fig. 1D). Inhibitory effects similar to those observed with letrozole were also seen on combining ENT with other AIs such as anastrozole and exemestane (data not shown).

Although cell growth was inhibited by the HDACi ENT (IC50 = 85.4 nmol/L) in a steroid-depleted medium, in the presence of E2 or Δ4Δ, cell growth was stimulated (IC50 = 2.8 μmol/L) because of activation of ERα when the ligand was provided. This counteracted the growth inhibition effect of ENT alone. Thus, the dose–response curve shifted to the right on addition of Δ4Δ (10 nmol/L) to the treatment medium (Fig. 1E). Growth stimulation was also seen when MDA-MB-231 cells were treated with ENT for 3 days and then treated with Δ4Δ at various concentrations. Δ4Δ caused mitogenic effects, suggesting that the estrogen produced by aromatase counteracted the inhibition due to ENT alone. A similar growth pattern is seen with ER-positive MCF-7Ca cells and Δ4Δ (Fig. 1F; refs. 8, 13).

**Mechanism of upregulation of aromatase**

*In vitro* chromatin immunoprecipitation (ChIP) assay was used to determine whether the aromatase gene was activated in ERα-dependent or -independent manner. MDA-MB-231 cells were treated with or without ENT for 24 hours, followed by E2 or Δ4Δ/letrozole for 3 hours. The cells were fixed and chromatin fragments were precipitated with acetyl histone H3 (Fig. 2A) or ERα antibody (Fig. 2B). Total histone H3 (Fig. 2C) served as a positive control and no antibody was included in the negative control (Fig. 2D). Treatment with ENT induced transcriptional activation of aromatase promoter, as evidenced by increased recruitment of acetyl histone H3. This recruitment was not affected by treatment with E2, Δ4Δ, or letrozole. In addition, ERα was not recruited at the aromatase promoter; consistent with this finding that blocking ER with ER downregulator did not affect the ENT-induced increase in aromatase. These results suggest that ENT activates aromatase in an ERα-independent and ligand-independent manner.
Figure 3. A, effect of ENT on the growth of MDA-MB-231 xenografts: MDA-MB-231 xenografts were grown in OVX athymic nude mice. Mice were treated with increasing doses of ENT and tumor volumes were plotted versus time. B, effect of doses of ENT on tumor and uterine weights of mice with MDA-MB-231 xenografts: tumor (left y-axis) and uterine (right y-axis) weights were measured at necropsy of above mice in A. C, effect of doses of ENT on ERα and aromatase protein expression in MDA-MB-231 xenografts: Western analysis of lysates of tumors from above mice in A; lane: 1, vehicle-treated control; 2, ENT (1 mg/kg/d); 3, ENT (2.5 mg/kg/d); 4, ENT (5 mg/kg/d); and 5, ENT (10 mg/kg/d). Blots show ERα, aromatase (CYP-19), and β-actin. D, effect of doses of ENT on aromatase activity of mice with MDA-MB-231 xenografts: Aromatase activity was measured by 3H2O release assay and corrected for total protein concentration (*, P < 0.05 vs. control). E, effect of doses of ENT on the mRNA expression of ERα, pS2, and aromatase (CYP-19) in MDA-MB-231 xenografts: Expression of mRNA was analyzed by RT-PCR. Lane: 1, control; 2, ENT (1 mg/kg/d); 3, ENT (2.5 mg/kg/d); 4, ENT (5 mg/kg/d); and 5, ENT (10 mg/kg/d). A representative gel image shows ERα and aromatase (CYP-19) and 18s ribosomal RNA (rRNA) as the loading control.
tumor growth. This increased growth effect is clearly seen in the estrogen-sensitive uteri of animals given ENT plus ΔΔ4A (Fig. 4B) compared with those of control mice (P < 0.01) and those treated with ENT alone (P < 0.05). Thus, although ENT alone increases ER and aromatase activity, there was no evidence of stimulatory effects on the uteri of these animals but only on the uteri of mice receiving ENT and the estrogen precursor ΔΔ4A (Fig. 4B).

In tumors, the mRNA levels of estrogen-regulated genes pS2 and PgR were higher in those treated with ENT plus ΔΔ4A (Fig. 4C). These results strongly support our findings that protein levels of ERα and aromatase in the tumors were upregulated by ENT (Fig. 3C and E). Activation of ERα by estrogen (converted from ΔΔ4A) induced transcription of pS2 and PgR. Consistent with these results, treatment with letrozole inhibited transcription of pS2 and PgR (Fig. 4C). Collectively, these results suggest that ENT converted triple-negative MDA-MB-231 xenografts into ER-positive and hormone-responsive tumors.

**Combining ENT with the AI letrozole inhibits tumor growth in vivo**

Mice bearing MDA-MB-231 xenografts (~150 mm³) were assigned to 4 groups; control (vehicle), ENT (2.5 mg/kg/d), po, letrozole (10 μg/d), or ENT plus letrozole. Except for the control group, all the mice received ΔΔ4A supplement. The mean tumor volume on day 0 was not statistically different across groups (P = 0.88). As shown in Figure 5A, the ENT plus letrozole markedly inhibited tumor growth compared with the control (P = 0.004), ENT (P = 0.009), or letrozole (P = 0.049) groups. At autopsy (Fig. 5B), the mean tumor weight of mice treated with ENT plus letrozole was significantly lower than the control (P < 0.001), ENT, or letrozole groups (P < 0.01). These results suggest that aromatase activity induced by ENT was blocked by letrozole, resulting in little or no estrogen production as confirmed by reduction in uterine weight (Fig. 5B). This resulted in almost complete inhibition of tumor growth.

Western blot analysis of the MDA-MB-231 tumors confirmed that in ENT-treated tumors, protein expression of ERα, PgR, and aromatase was upregulated (Fig. 5C). The tumors of mice treated with ENT plus letrozole had increased levels of aromatase protein compared with controls and ERα/PgR levels were higher than those with letrozole alone. Furthermore, ENT treatment significantly increased intratumoral aromatase activity in comparison with controls (P < 0.0001), which was markedly inhibited by treatment with ENT plus letrozole compared with ENT alone (Fig. 5D).

Similar responses were seen in tumors of a second ER-negative cell line, Hs578T (Fig. 6), and in LTLT-Ca cells (refs. 7, 12, 24; Supplementary Fig. 3). Tumors of mice treated with ENT plus letrozole had significantly smaller tumors (P < 0.001) than control, ENT plus ΔΔ4A, and ΔΔ4A plus letrozole groups (Fig. 5).
IHC of treated tumors

Histopathologic analysis of hematoxylin and eosin-stained sections of the MDA-MB-2331 tumors (Supplementary Fig. 4) revealed a highly necrotic center in the tumors from the ENT plus letrozole-treated mice, confirming the effectiveness of the combination treatment on inhibiting the growth of the tumors. Furthermore, IHC revealed that ERα was expressed in the epithelial cells (Supplementary Fig. 4). These results confirmed previous findings that ENT treatment results in expression of ERα, rendering the MDA-MB-231 cells and tumors responsive to growth inhibitory effects of letrozole. In fact, tumors treated with the combination had fewer cells that stained positive for the proliferation marker Ki67 than the control tumors (Supplementary Fig. 4).

Combination of ENT plus letrozole inhibits lung colonization in metastasis model

To test the efficacy of this combination in preventing the outgrowth of tumor foci in the lung as a model for metastasis, mice were injected with MDA-MB-231 cells via the tail vein.
Colonization of cells into the lungs resulted in visible or microscopic tumor foci (Fig. 7A and B). All 6 of 6 mice in the control group showed an average of 60 visible metastases and 15 micrometastases per animal. Five of 6 mice in the ENT group had visible metastases (C24 on average) and micrometastases (C24). Three of 6 mice in the letrozole group had visible metastases (C24) and micrometastases (C24). In contrast, only 1 of 6 mice treated with ENT plus letrozole showed 1 visible and 2 micrometastases in the lungs. These results provide strong evidence that the combination of ENT plus letrozole markedly inhibits growth of tumor foci in the lungs.

**Discussion**

ERα-negative breast cancers remain difficult to manage and treatment options are limited to chemotherapy because the tumors are more aggressive and resistant to endocrine therapy. Genetic alternations such as mutations, LOH, or homozygous deletions are rare (6, 25). Studies conducted by Davidson and colleagues suggested that the loss of ERα protein expression is the result of the hypermethylation of the CpG islands within the ERα promoter (5, 26). Deacetylated histones were associated with the inactive ERα promoter in MDA-MB-231 cells, whereas acetylated histones were associated with the active ERα promoter in MCF-7 cells (25). Treatment with HDACis reversed repression of ERα in MDA-MB-231 cells (27). In this article, we show that the HDACi ENT was effective in causing expression of ERα and aromatase, the enzyme that is key in the production of estrogen. Targeting the upregulated aromatase, using letrozole, caused reduced cell viability in vitro and tumor regression in vivo. Our studies investigated both biochemical and biological consequences of
**Entinostat-Letrozole Therapy of ER-Negative Breast Cancer**

Entinostat-Letrozole Therapy of ER-Negative Breast Cancer

![Graph](image)

Figure 7. Effect of treatment with ENT and letrozole alone or in combination on colonization of MDA-MB-231 cells in the lungs of mice. MDA-MB-231 cells were injected into the tail vein. The mice were treated with after 3 weeks with vehicle, ENT plus Δ^4^-A, Δ^4^-A plus letrozole, or combination, for 6 weeks. Visible or micrometastatic foci were quantitated at autopsy. A, the combination of ENT (ENT) plus letrozole produced significantly fewer visible lung foci than control (*, P = 0.002) and ENT (†, P = 0.02). B, mice treated with a combination of ENT plus letrozole had significantly fewer micrometastases than control (†, P = 0.0269) and ENT (†, P = 0.038).

these treatments and provide a strong rationale for use of AIs in combination with HDACis for the treatment of ER-negative breast cancers.

Several studies have now confirmed that gene silencing by methylation involves generation of inactive chromatin structure, characterized by deacetylated histones (4, 26, 27). The HDACs deacetylase lysine groups of histones H3 and H4, allowing ionic interactions between positively charged lysine residues on histones and negatively charged DNA. This results in compaction of the nucleosomes, which prevents transcription (3, 4). An endogenous interaction exists between HDAC 1 and ERα in the absence of estrogen in breast cancer cells (28). HDAC2 and 3 have been indicated to associate with ERα-regulated genes such as c-Myc and cathepsin D (27). ENT was used in this study because of its specificity for class I HDACs such as HDAC 1, 2, and 3. ENT also exhibited a long half-life (~100 hours) and favorable pharmacodynamic effects in humans (29, 30).

Our novel findings show that HDACi ENT can lead to an increase in functional tumoral aromatase activity and an increase in ERα-mediated transcription (pS2 upregulation), both *in vitro* and *in vivo*. Our previous study with letrozole-resistant LTLT-Ca cells showed that upregulation of ERα led to activation of aromatase gene transcription in a ligand-dependent manner (24). However, in MDA-MB-231 cells treated with ENT, aromatase was upregulated in ERα-independent manner.

Among the 3 HDACis tested, ENT, SAHA, and BA. ENT was the most potent. These effects were not specific to just one cell line, MDA-MB-231. *In vitro* studies with 2 other ERα-negative cell lines (SKBr-3 and HS57ST) confirmed the results obtained with MDA-MB-231. On the basis of these findings, we hypothesized that treatment with an HDACi would convert ER-negative tumors to ER-positive tumors, thereby rendering them sensitive to estrogens and, consequently, the inhibitory effects of AIs. Treatment of xenografts with MDA-MB-231 (Fig. 5A) and HS57ST tumors (Fig. 6) confirmed this hypothesis. In initial dose finding studies, reduction in tumor growth was observed in OVX mice treated with ENT alone (Fig. 3A). However, when the mice were supplemented with Δ^4^-A to produce estrogen, tumor growth was slightly stimulated, thus negating the inhibitory effect of ENT.

The induction of aromatase in the tumor leads to the production of estrogen via aromatization of Δ^4^-A. This results in the activation of ERα and transcription of ERα-regulated genes, leading to tumor growth, thereby counteracting the tumor inhibitory effect of ENT (Figs. 1E and F and 3A). In this setting, combining the AI letrozole with ENT inhibited production of estrogen. This resulted in inhibition of tumor growth and lung metastases.

ERα-positive cancer cell lines respond to HDACi differently. In these cells, in contrast to our findings in ERα-negative cell lines, Chen and colleagues have shown that the HDACi LBH589 specifically inhibits aromatase activity and downregulates gene and protein expression through suppression of promoters *L3/PIL* (31). SAHA acts in a similar manner in MCF-7 and BT-474 cells (32). SAHA downregulates ERα through hyperacetylation of HSP-90, a chaperone protein that maintains the stability of ERα (32). HDAC6 is HSP90 deacetylase and inhibition of HDAC6 is responsible for HDACi-mediated HSP90 inhibition (33, 34). This complex regulation of ERα by HDACi in ER-positive versus ER-negative breast cancer warrants further investigation. There is also some evidence of partial restoration of functional ERα in cells that have lost ERα and PgR expression as a result of acquired resistance to endocrine therapy (35, 36). In this model system, expression of PgR could not be restored with reexpression of ERα.

The detailed molecular mechanism of the conversion of MDA-MB-231 cells from hormone-independent to hormone-dependent cells expressing ER and aromatase is unknown at this time. However, it suggests phenotypic plasticity of the...
cells (37, 38) that enables them to adapt (11–13, 24) to changes in their microenvironment. Further studies are needed to elucidate the precise mechanisms underlying this phenomenon.

In conclusion, our study using both biological and biochemical assays shows that the HDACi ENT increases both ERα and aromatase expression and activity, thereby converting ER-negative tumors to ERα-positive tumors. The breast cells are now sensitive to the growth stimulatory effects of estrogens synthesized locally by the aromatization of Δ4A in the tumor. Thus, when the AI letrozole was combined with the HDACi, the ERα-expressing tumors were deprived of estrogen. This resulted in suppression of tumor growth. Furthermore, the combination treatment was also effective in inhibiting tumor cell colonization in the lungs. This novel approach could potentially provide a new treatment strategy for the management of ERα-negative breast cancer.

References

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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