HDAC Inhibitor SNDX-275 Induces Apoptosis in erbB2-Overexpressing Breast Cancer Cells via Down-regulation of erbB3 Expression

Xiaoping Huang,1 Lizhi Gao,1 Shuiliang Wang,1 Choon-Kee Lee,2 Peter Ordentlich,3 and Bolin Liu1

1Department of Pathology and 2The Myeloma and Amyloidosis Program, Department of Medicine, University of Colorado Denver School of Medicine, Aurora, Colorado; and 3Syndax Pharmaceuticals, Inc., San Diego, California

Abstract
Breast cancer is a highly heterogeneous disease with distinct histologic subtypes. Targeted therapies such as endocrine therapy and growth factor receptor inhibitors have had a significant impact on the treatment of metastatic breast cancer patients. Unfortunately, resistance to these agents eventually occurs, and currently represents a significant clinical problem in the management of breast cancers. Inhibitors of histone deacetylases (HDACi) exhibit anticaner activity in a variety of tumor cell models and have been shown to target mechanisms of resistance to a number of targeted agents. It is unclear, however, if there are specific breast cancer subtypes for which an HDACi may be more or less effective. Here, we report that the class I isoform-selective HDACi entinostat (SNDX-275) preferentially inhibits cell proliferation/survival and inactivates downstream signaling in erbB2-overexpressing compared with basal breast cancer cells. SNDX-275 reduces the levels of both erbB2 and erbB3, as well as significantly decreases P-erbB2, P-erbB3, P-Akt, and P-MAPK in erbB2-overexpressing cells. Additionally, SNDX-275 promotes apoptosis and induces cell cycle arrest predominantly at G1 phase in erbB2-overexpressing cells, whereas SNDX-275 mainly induces G2-M arrest in basal breast cancer cells. The cellular bias of SNDX-275 is shown to be related partly to the levels of erbB3 expression that directly impact the ability of SNDX-275 to inhibit proliferation/survival of the erbB2-overexpressing breast cancer cells. These findings show that SNDX-275 may be developed as a novel therapeutic agent to treat breast cancers with coexpression of both erbB2 and erbB3. [Cancer Res 2009;69(21):8403–11]

Introduction
Breast cancer is a highly heterogeneous disease with distinct histologic subtypes. Gene microarray-profiling studies of invasive breast carcinomas have identified five distinct subtypes [luminal A, luminal B, erbB2-overexpressing, basal (or “triple negative”), and normal breast like] that are associated with different clinical outcomes (1–3). Although luminal A and normal breast-like subtypes have a better prognosis, erbB2-overexpressing and basal breast cancers have the least favorable prognosis. Recent advances in our understanding of breast cancer biology have led to improved patient survival with target therapies, for example, antiestrogen agents (tamoxifen) in the treatment of estrogen receptor (ER)–positive breast cancers (4); anti-erbB2 antibody (trastuzumab or Herceptin) for breast cancer patients with erbB2 amplification and/or overexpression (5). Trastuzumab has been successfully used in metastatic breast cancer patients with erbB2-overexpressing tumors as monotherapy (6) and in combination with chemotherapy (5, 7); however, both primary and acquired resistances to trastuzumab are common (8), and currently represent a significant clinical problem. It is critical to explore novel therapies targeting erbB receptors for breast cancer treatment.

Alterations in chromatin structure by histone modification play a vital role in regulation of gene transcription. Acetylation of core nucleosomal histones is regulated by the opposing behaviors of histone acetyltransferases and histone deacetylases (HDAC; refs. 9, 10). Studies indicate that deregulation of acetylation and deacetylation plays an important role in aberrant gene expression in human cancers (11, 12). Although targeting any component, such as DNA, transcription factor, or modifying enzyme, which contributes to aberrant gene expression in tumorigenesis might be a good therapeutic strategy in cancer treatment, HDACs represent relatively easier tractable enzymes. Thus, HDACs have recently become promising molecular targets, and a variety of inhibitors of HDACs are actively under investigation as potential anticancer agents (13, 14). HDAC inhibitors (HDACi) have been found to possess anticancer activity in a variety of tumor cell models via influencing cell cycle progression, apoptosis, differentiation, and tumor angiogenesis (15, 16).

HDACis, differing in respect to their antitumor activity, toxicity, stability, and specificity, can be divided into several structural classes (15, 17, 18). SNDX-275 (entinostat; formerly MS-275) is a synthetic benzamide derivative class I–selective HDACi. It inhibits cancer cell growth accompanied by an induction of the cyclin-dependent kinase (CDK) inhibitor p21WAF1, which is one of the most commonly induced genes by HDACi (19). SNDX-275 exhibits both in vitro and in vivo activities against various cancer types, including colorectal, lung, ovary, and pancreatic cancers (20), pediatric solid tumors (21), leukemia (20, 22–24), and prostate cancer (25). In breast cancer, SNDX-275 has been shown to inhibit cell proliferation and/or promote apoptosis by up-regulating expression of transforming growth factor β type II receptor (26, 27) or tumor necrosis factor–related apoptosis-inducing ligand (28, 29), or inducing degradation of DNA methyltransferase I (30). Thus far, little is known about the specificity of an HDACi against breast cancer subtypes. A recent report indicates that class II HDACis may exert particular antiproliferative effects in ER positive breast cancer cells (31). However, there is no report indicating whether SNDX-275 has any preference against different subtypes of breast cancers, and it is unclear whether SNDX-275 might target erbB receptors, such as epidermal growth factor receptor (EGFR), erbB2, and erbB3, in breast cancer cells. In the current studies, we have investigated the potential therapeutic efficacy of SNDX-275 on erbB2-overexpressing and basal breast cancer cells, focusing on the effects of
SNDX-275 on the activation and expression of erbB receptors and their downstream signaling pathways.

**Materials and Methods**

**Reagents and antibodies.** MISSION Nontarget shRNA, which does not target human and mouse genes, control vector (pLKO.1-ConshRNA), was obtained from Sigma Co. The lentiviral vector pLKO.1-puro containing a human-specific erbB3 shRNA (pLKO.1-ErbB3shRNA) and lentivirus packaging plasmids pCMV-MSV and pCMV-ΔA.9 were kindly provided by Dr. Haihui Gu (Department of Pathology, University of Colorado Denver School of Medicine, Aurora, CO). SNDX-275 (1-(2-Aminophenyl)-4-

**Flow cytometry analysis.** Cell cycle distribution was analyzed by flow cytometric analysis as described (34). In brief, cells grown in 100-mm culture dishes were harvested by trypsinization and fixed with 70% ethanol. Cells were stained for total DNA content with a solution containing 50 μg/mL propidium iodide and 100 μg/mL RNase I in PBS for 30 min at 37°C. Cell cycle distribution was then analyzed at the Core Facility of University of Colorado Cancer Center with a FACSscan flow cytometer (BD Biosciences).

**Quantification of apoptosis.** An apoptosis ELISA kit (Roche Diagnostics Corp.) was used to quantitatively measure cytoplasmic histone-associated DNA fragments (mono- and oligonucleosomes) as previously reported (33, 34).

**Western blot analysis.** Protein expression levels were determined by Western blot analyses as described (32, 33). Briefly, cells were lysed in a buffer containing 50 mmol/L Tris (pH 7.4), 50 mmol/L NaCl, 0.5% NP40, 50 mmol/L NaF, 1 mmol/L Na3VO4, 1 mmol/L phenylmethylsulfonyl fluoride, 25 μg/mL leupeptin, and 25 μg/mL aprotinin. The lysates were centrifuged at full speed for 20 min and the supernatants were collected for protein concentration determination by the Coomassie Plus protein assay reagent (Pierce Chemical Co.). Equal amounts of cell lysates were subjected to Western blot analysis with specific antibodies as described in the figure legends.

**Statistical analysis.** Statistical analyses of the experimental data were performed using a two-sided Student’s t test. Significance was set at a P value of <0.05.

**Results**

SNDX-275 selectively inhibits cell proliferation/survival of erbB2-overexpressing breast cancer cells in vitro. To explore whether SNDX-275 might be a potential therapeutic agent against erbB2-overexpressing and basal breast cancers, we investigated its antiproliferative/antisurvival activities in erbB2-overexpressing (SKBR3, BT474, MDA-MB-453) and basal (MDA-MB-468, BT20, MDA-MB-231) breast cancer cell lines. SNDX-275 inhibited proliferation of all cells in a dose-dependent manner. The erbB2-overexpressing cells were much more sensitive than basal cells to SNDX-275-induced growth inhibition (Fig. 1A). The IC50 values were 0.4 to 1.2 μmol/L for erbB2-overexpressing cells, and 2.5 to 4.5 μmol/L for basal cells. As a class I HDACi, SNDX-275 showed equal efficacy in inducing histone H3 acetylation in both erbB2-overexpressing and basal breast cancer cells (Fig. 1B and C); however, SNDX-275 exhibited different effects on the downstream signaling pathways. Although SNDX-275 significantly decreased the levels of P-Akt in SKBR3, BT474, and MDA-MB-453 cells in a dose-dependent manner (Fig. 1B), it was less effective on modulation of P-Akt in MDA-MB-468, BT20, and MDA-MB-231 cells (Fig. 1C). SNDX-275 also reduced the levels of P-MAPK in SKBR3 and MDA-MB-453 cells, but had no significant effects on P-MAPK in all basal breast cancer cells tested. Thus, SNDX-275 preferential inhibition of cell proliferation/survival of erbB2-overexpressing cells correlated with inactivation of the downstream signaling Akt and/or MAPK.

SNDX-275 blocks cell cycle progression and selectively induces caspase-dependent apoptosis in erbB2-overexpressing breast cancer cells. To determine the molecular mechanisms by which erbB2-overexpressing breast cancer cells were more sensitive to SNDX-275-mediated inhibitory effects, we first studied whether SNDX-275 might induce apoptosis in these breast cancer cells. Apoptosis-specific ELISA revealed that SNDX-275 dramatically induced apoptosis in SKBR3, BT474, and MDA-MB-453 cells,
but only marginally promoted apoptosis in MDA-MB-468, BT20, and MDA-MB-231 cells (Fig. 2A). The induction fold of apoptosis was 8.25 for SKBR3, 18.25 for BT474, and 9.98 for MDA-MB-453 cells. In contrast, the fold of apoptosis induction was significantly less in all three basal lines (2.4 for MDA-MB-468, 3.5 for BT20, and 3.3 for MDA-MB-231 cells) compared with that of SKBR3 cells (P < 0.001). Furthermore, Western blot analyses indicated that treatment with SNDX-275 in erbB2-overexpressing, but not basal, breast cancer cells induced PARP cleavage and activation of both caspase-8 and caspase-3 evidenced by the increases of cleaved caspase-8 and caspase-3 (Fig. 2B), suggesting that SNDX-275 induced caspase-dependent apoptosis in erbB2-overexpressing breast cancer cells. Next, we investigated whether SNDX-275 might have different modulation on cell cycle progression. SNDX-275 predominantly induced G1 arrest and exhibited a less significant effect on G2-M arrest in SKBR3, BT474, and MDA-MB-453 cells, whereas in the basal cells SNDX-275 mainly arrested the cells at G2-M phase (Fig. 3A). These data correlated well with the Western blot analyses on several key molecular markers regulating cell cycle progression. Consistent with a previous report showing that the CDK inhibitor p21^{waf1} is one of the most commonly induced genes by HDACi (19), we did discover a significant induction of p21^{waf1} by SNDX-275 (Fig. 3B and C), which may contribute to the cell cycle G1-M arrest we observed in all cells. Cyclin D1 and E2F1 are two important positive regulators at the G1-S checkpoint of cell cycle. Treatment with SNDX-275 reduced the levels of both cyclin D1 and E2F1 in a dose-dependent manner only in the erbB2-overexpressing cells (Fig. 3B). E2F1 reduction was also found in one of the basal cell lines (MDA-MB-468), which might explain why SNDX-275 induced minor G1 arrest in MDA-MB-468 cells (Fig. 3C and A). Collectively, our results show that SNDX-275 selectively induces caspase-dependent apoptosis in erbB2-overexpressing cells, and blocks cell cycle progression in both erbB2-overexpressing and basal breast cancer cells.

**SNDX-275 treatment results in down-regulation of both erbB2 and erbB3, but not EGFR in breast cancer cells.** Basal breast cancer cells lack expression of ER, progesterone receptor, and erbB2, but often overexpress EGFR and/or cytokeratin 5/6 (1). In contrast, erbB2-overexpressing breast cancer cells generally have little EGFR expression, but frequently coexpress with erbB3 (35, 36). To study why these two subtypes showed distinct sensitivity to SNDX-275, we concentrated our following experiments on erbB receptors, and wondered whether this HDACi might possess particular effects on the expression and/or activation of the erbB receptors. In erbB2-overexpressing cells, the levels of P-erbB2, erbB2, P-erbB3, and erbB3 were all significantly reduced in a dose-dependent manner upon SNDX-275 treatment (Fig. 4A). In contrast, SNDX-275 had no significant effects on the levels of both

---

**Figure 1.** SNDX-275 preferably inhibits proliferation/survival of erbB2-overexpressing compared with basal breast cancer cells. A, the indicated cells were plated onto 96-well plates with complete medium (DMEM/F12, 10% FBS). After 24 h, the medium was replaced with control medium (DMEM/F12, 0.5% FBS) or same medium containing the indicated concentrations of SNDX-275 for another 72 h. The percentages of surviving cells from each cell line relative to controls, defined as 100% survival, were determined by reduction of MTS. Points, mean of three independent experiments; bars, SD. B and C, cells treated with the indicated concentrations of SNDX-275 for 24 h were collected and subjected to Western blot analyses of P-Akt, Akt, P-MAPK, MAPK, Acetyl-Histone H3, Histone H3, or β-actin.
P-EGFR and EGFR in those basal cells, although at the highest concentration (5 μmol/L) we tested, SNDX-275 marginally decreased the levels of P-EGFR and EGFR in MDA-MB-468 cells and MDA-MB-231 cells, respectively (Fig. 4B). It seemed SNDX-275 preferably targeted erbB2/erbB3 receptors rather than EGFR. To further support this hypothesis, additional experiments were performed to evaluate whether SNDX-275 might have any effects on EGFR expression in those erbB2-overexpressing cells. Although EGFR expression was clearly observed in the control cells, it was undetectable with 1-minute exposure and showed fine signals with a longer exposure time (15 minute) in SKBR3, BT474, and MDA-MB-453 cells (Fig. 4C), confirming that the erbB2-overexpressing breast cancer cells indeed have little EGFR expression. Nonetheless, the expression levels of EGFR remained unchanged upon SNDX-275 treatment in all three lines (Fig. 4C). Thus, the discrimination effects on erbB receptors by SNDX-275 might account for its distinct activities on the downstream signaling Akt and MAPK.

The class I HDAC contains HDAC1, HDAC2, HDAC3, and HDAC8 (17, 37, 38). SNDX-275 preferentially inhibits HDAC1 compared with HDAC3, and has little or no effect on HDAC8 (39). We next explored the potential effects of SNDX-275 on HDAC1, HDAC2, and HDAC3 in these breast cancer cells. SNDX-275 treatment had no effect on HDAC3, but resulted in gradually reduction of HDAC1 in SKBR3, BT474, and MDA-MB-453 cells. Although similar reduction of HDAC2 was observed in SKBR3 and MDA-MB-453 cells, the expression levels of HDAC2 in BT474 cells remained unchanged upon SNDX-275 treatment (Fig. 4D). In contrast, SNDX-275 did not reduce the expression levels of HDAC1, HDAC2, or HDAC3 in all three basal breast cancer cells. Thus, there is a correlation between SNDX-275–induced HDAC1 and HDAC2 reduction and its inhibitory effects on erbB2/erbB3 and their downstream signaling.

Elevated expression of erbB3 activates Akt signaling and attenuates SNDX-275–induced apoptosis in erbB2-overexpressing breast cancer cells. To determine whether down-regulation of erbB3 is required for SNDX-275–induced apoptosis in erbB2-overexpressing cells, exogenous expression of erbB3 via transient transfection was performed in SKBR3, BT474, and MDA-MB-453 cells. Transfection with pDsRed-erbB3, compared with transfection with the control vector, significantly abrogated SNDX-275–mediated antiproliferative/antisurvival effects (Fig. 5A). At the molecular level, elevated expression of erbB3 completely reversed SNDX-275–mediated reduction in P-erbB3 and P-Akt, but had little effect on the reduction of P-erbB2 and P-MAPK by SNDX-275 (Fig. 5B, left). Moreover, forced expression of erbB3 also decreased the levels of cleaved PARP, caspase-8, caspase-3–induced by SNDX-275.

Figure 2. SNDX-275 induces apoptosis, PARP cleavage, and activation of caspases in erbB2-overexpressing breast cancer cells. Cells cultured with DMEM/F12 (0.5% FBS) in the presence or absence of SNDX-275 for 24 h were collected and subjected to apoptosis ELISA (A). Columns, mean of three independent experiments; bars, SD. * P < 0.001 versus SNDX-275–induced apoptosis in SKBR3 cells (#). B, Western blot analyses of PARP, caspase-8, caspase-3, or β-actin.

Figure 3. SNDX-275 blocks cell cycle progression and modulates expression of several key cell cycle regulators. A, the indicated cells cultured with DMEM/F12 (0.5% FBS) in the presence or absence of SNDX-275 for 24 h were harvested and subjected to flow cytometry analysis of cell cycle distribution. Bar graph, the percentage of cells in G1, S, or G2/M phase of the cell cycle. Data are representative of at least three independent experiments. B and C, human breast cancer cells treated with the indicated concentrations of SNDX-275 for 24 h were collected and subjected to Western blot analyses of cyclin D1, E2F1, p27Kip1, p21Waf1, or β-actin.
(Fig. 5B, right), suggesting a weakened apoptosis. Specific apoptosis ELISA further confirmed that transfection with pDsRed-erbB3, not the control vector, significantly reduced SNDX-275–induced apoptosis (Fig. 5C). Collectively, elevated expression of erbB3 attenuates SNDX-275–induced apoptosis via activation of Akt in erbB2-overexpressing breast cancer cells.

Specific knockdown of erbB3 expression enhances SNDX-275–induced apoptosis in erbB2-overexpressing breast cancer cells. We next investigated whether knockdown of erbB3 expression may have synergistic antiproliferative/antisurvival effects with SNDX-275. Specific knockdown of erbB3 was achieved with a lentiviral system containing human erbB3 shRNA. As shown in Fig. 6A, erbB3 shRNA, but not control shRNA, dramatically decreased erbB3 expression levels in SKBR3, BT474, and MDA-MB-453 cells. In contrast, erbB2 levels remained unchanged in all cells, suggesting that the erbB3 shRNA was specific and efficient. Specific knockdown of erbB3 did not induce apoptosis. Consistent with previous data, SNDX-275 treatment alone reduced erbB3 and erbB2 expression; induced PARP, caspase-8, and caspase-3 cleavage (but no caspase-9 cleavage); and promoted apoptosis in all three cell lines. Importantly, combination of erbB3 knockdown and SNDX-275 treatment further increased the levels of cleaved PARP, caspase-8, caspase-3, and induced caspase-9 cleavage (Fig. 6D), and significantly accelerated apoptosis compared with either erbB3 knockdown or SNDX-275 treatment alone (Fig. 6B). On the downstream signaling pathways, either erbB3 knockdown or SNDX-275 treatment alone reduced the levels of P-Akt, and their combination further reduced P-Akt levels. Although SNDX-275 treatment alone decreased P-MAPK in SKBR3 and MDA-MB-453, but not BT474 cells, which was consistent with previous data (Fig. 1B), specific knockdown of erbB3 alone decreased P-MAPK only in BT474 cells. The combination of erbB3 knockdown and SNDX-275 treatment had no further significant effects on P-MAPK (Fig. 6A). Although elevated expression of erbB3 via transfection attenuated SNDX-275–induced histone H3 acetylation (Fig. 5B), erbB3 knockdown strikingly increased the levels of histone H3 and its acetylation upon SNDX-275 treatment. Thus, specific knockdown of erbB3 expression enhances SNDX-275–induced apoptosis and inactivation of the downstream signaling Akt in erbB2-overexpressing breast cancer cells.

Figure 4. SNDX-275 differentially modulates the expression and activation of erbB receptors in breast cancer cells. A, SKBR3, BT474, and MDA-MB-453 cells treated with the indicated concentrations of SNDX-275 for 24 h were collected and subjected to Western blot analyses of P-erbB2, erbB2, P-erbB3, erbB3, or β-actin. B, MDA-MB-468, BT20, and MDA-MB-231 cells treated with the indicated concentrations of SNDX-275 for 24 h were collected and subjected to Western blot analyses of P-EGFR, EGFR, or β-actin. C, SKBR3, BT474, and MDA-MB-453 cells treated with the indicated concentrations of SNDX-275 for 24 h along with BT20 and MDA-MB-231 cells were collected and subjected to Western blot analyses of EGFR or β-actin. D, the cells treated with the indicated concentrations of SNDX-275 for 24 h were collected and subjected to Western blot analyses of HDAC1, HDAC2, HDAC3, or β-actin.
Discussion

Coexpression of erbB family members is common in human breast cancer. Novel strategies that simultaneously target multiple erbB receptors have drawn great interest. Agents that inhibit both EGFR and erbB2 tyrosine kinase activities have been developed (40). One of these new agents, lapatinib (GlaxoSmithKline), has been approved by Food and Drug Administration to treat metastatic breast cancer patients whose tumors overexpress erbB2 (41). However, only a minority of erbB2-altered breast cancers have coexistent overexpression of EGFR. Most metastatic breast cancers show expression for either EGFR or erbB2, and less often for both (42). In contrast, coexpression of erbB3 and erbB2 is frequently observed in breast cancers (35) and breast cancer cell lines (36). It has been reported that erbB2 requires erbB3 to promote breast cancer cell proliferation (43), and erbB3 plays an important role in erbB2-altered breast cancers (44). Our recent studies indicate that expression of erbB3 contributes to erbB2-mediated tamoxifen resistance (33). Thus, novel strategies or agents simultaneously targeting both erbB3 and erbB2 may have a broader impact on treatment of breast cancer.

In an attempt to identify novel therapeutic agents for erbB2-overexpressing and basal breast cancers, we have discovered that the class I HDACi SNDX-275 exhibits a greater efficacy against erbB2-overexpressing cells. At its clinically achievable range (≤1 μmol/L; ref. 45), SNDX-275 strongly inhibits proliferation of erbB2-overexpressing breast cancer cells (Fig. 1A), which is accompanied by significant reduction of cyclin D1 and E2F1 (Fig. 3B). Mechanistically, SNDX-275 inactivates the Akt and/or MAPK signaling via down-regulation of both erbB3 and erbB2 (Figs. 1B and 4A). In contrast, SNDX-275 has little effect on EGFR. More importantly, elevated expression of erbB3 abrogates and specific knockdown of erbB3 enhances SNDX-275-mediated inactivation of Akt and Akt-induced apoptosis in erbB2-overexpressing breast cancer cells. These significant findings not only confirm that erbB3 is required for erbB2-mediated breast cancer cell proliferation and survival, but also suggest that targeting erbB3 may be an excellent therapeutic
strategy against breast cancers with coexpression of erbB3 and erbB2.

Interestingly, we have repeatedly observed that SNDX-275 specifically reduced the protein levels of endogenous erbB3, but not the exogenous erbB3 via transient transfection, and therefore, it had no effect on the P-Akt levels increased by elevated expression of erbB3 (Fig. 5B). The similar discrimination effects of SNDX-275 on endogenous and exogenous erbB2 were also found (data not shown). These results suggest that the mechanisms of SNDX-275–mediated reduction of erbB2 and erbB3 may be independent of protein degradation, and it might modulate expression of certain microRNAs that specifically target both erbB2 and erbB3. We are currently testing this novel hypothesis.

It has been reported that LAQ824 and suberoylanilide hydroxamic acid (SAHA) are able to down-regulate erbB2 in SKBR3 and BT474 cells via acetylation of heat shock protein (hsp) 90 (46, 47). This hsp90 acetylation reduces the chaperone association with its client erbB2, shifts the binding of erbB2 from hsp90 to hsp70, and subsequently promotes ubiquitylation-dependent degradation of erbB2 (46, 47). As two broad spectrum HDACis, both LAQ824 and SAHA inhibit the activity of HDAC6, which is the major HDAC that modulates hsp90 activity (48, 49). However, as a specific class I HDACi, SNDX-275 has no effect on HDAC6 (17). Thus, SNDX-275–mediated reduction of erbB2 protein is through an HDAC6-independent mechanism. Although LAQ824 slightly reduces the levels of erbB3 (47), it remains unknown about the activity of SAHA toward erbB3 in breast cancer cells. To the best of our knowledge, SNDX-275 is the first agent with a strong activity of dual-targeting both erbB3 and erbB2 in breast cancer cells. Consistent with previous reports (46, 47), we have found that SNDX-275 induces cell

Figure 6. Specific knockdown of erbB3 expression enhances SNDX-275–induced inhibition of downstream signaling and apoptosis in erbB2-overexpressing breast cancer cells. SKBR3, BT474, and MDA-MB-453 were infected with lentivirus containing either ConshRNA or ErbB3shRNA. After 24 h, virus-infected cells were selected with puromycin (1 μg/mL) for 48 h, and then cultured with DMEM/F12 (0.5% FBS) in the presence or absence of SNDX-275 for 24 h. Cells were collected and subjected to Western blot analyses of erbB3, erbB2, PARP, caspase-8, caspase-9, caspase-3, P-Akt, Akt, P-MAPK, MAPK, Acetyl-Histone H3, Histone H3, or β-actin (A) or apoptosis ELISA (B).
cycle G2–M arrest in SKBR3, BT474, and MDA-MB-453 cells (Fig. 3A). Nonetheless, SNDX-275 predominantly arrests the cells at G1 phase, which is associated with a significant reduction in cyclin D1 and E2F1 (Fig. 3B). LAQ824 and SAHA do not arrest cells at G1 phase (46, 47), suggesting that these two HDACis might not affect the expression of cyclin D1 and E2F1 in SKBR3 and BT474 cells. Because of their ability to down-regulate erbB2, both LAQ824 and SAHA show synergistic cytotoxic effects against breast cancer with erbB2 amplification and/or overexpression when combined with trastuzumab or other drugs (46, 47). Because SNDX-275 dual-targets erbB3 and erbB2, and results in significant inactivation of Akt and/or MAPK, it should have a better synergism with trastuzumab and other chemotherapeutic agents. Indeed our recent studies indicate that SNDX-275 and trastuzumab exhibit synergistic antiproliferative/antisurvival activities in SKBR3 and BT474 cells. It will be interesting to explore whether SNDX-275 also has synergistic anticancer activities with another erbB2-targeted therapy, lapatinib, and if SNDX-275 can overcome trastuzumab resistance, and enhance trastuzumab-mediated growth inhibition in those trastuzumab-resistant cells.

Up-regulation of tumor necrosis factor–α-related apoptosis-inducing ligand has been reported as one of the molecular mechanisms by which HDACi, including SNDX-275, induces apoptosis in breast cancer cells (28, 29). Those studies were mainly performed in ER-positive and basal breast cancer cells with higher concentrations of SNDX-275. It is not known whether SNDX-275 can also induce tumor necrosis factor–α–related apoptosis-inducing ligand expression in erbB2-overexpressing breast cancer cells. To date, the molecular mechanisms of SNDX-275–induced down-regulation of erbB3 remain unclear. Studies on such molecular mechanisms may provide new avenues to identify novel strategies targeting erbB3 and the downstream signaling, which may enhance erbB2-targeted therapies in breast cancer patients whose tumors have co-expression of both erbB3 and erbB2.

In summary, our results show that the class I HDACi SNDX-275 preferably inhibits cell proliferation and induces apoptosis of erbB2-overexpressing breast cancer cells via down-regulation of erbB3 expression and inactivation of the Akt and/or MAPK signaling. SNDX-275 treatment causes cell cycle arrest predominantly at G1 phase in erbB2-overexpressing cells associated with reductions of both cyclin D1 and E2F1 and an up-regulation of p21waf1. These data suggest that SNDX-275 may be developed as a novel therapeutic agent to treat breast cancers with coexpression of both erbB3 and erbB2.

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

Acknowledgments
Received 6/11/09; revised 8/12/09; accepted 8/27/09; published OnlineFirst 10/13/09.
Grant support: This work was supported in part by a research grant from Susan G. Komen for the Cure (B. Liu).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank Dr. Hailiu Gu for the pl.KO.1-ErbB3ShRNA expression vector and lentivirus packaging plasmids.

References

Cancer Research 2009;69: (21). November 1, 2009
www.aacrjournals.org


Published OnlineFirst October 13, 2009; DOI: 10.1158/0008-5472.CAN-09-2146

Downloaded from cancerres.aacrjournals.org on April 12, 2017. © 2009 American Association for Cancer Research.
HDAC Inhibitor SNDX-275 Induces Apoptosis in erbB2-Overexpressing Breast Cancer Cells via Down-regulation of erbB3 Expression

Xiaoping Huang, Lizhi Gao, Shuiliang Wang, et al.


Updated version Access the most recent version of this article at: doi:10.1158/0008-5472.CAN-09-2146

Cited articles This article cites 49 articles, 25 of which you can access for free at: http://cancerres.aacrjournals.org/content/69/21/8403.full.html#ref-list-1

Citing articles This article has been cited by 1 HighWire-hosted articles. Access the articles at: /content/69/21/8403.full.html#related-urls

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.