Chemical Therapeutics

Potential Advantages of CUDC-101, a Multitargeted HDAC, EGFR, and HER2 Inhibitor, in Treating Drug Resistance and Preventing Cancer Cell Migration and Invasion

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Abstract

CUDC-101 is a novel, small-molecule, anticancer agent targeting histone deacetylase (HDAC), EGF receptor (EGFR), and HER2. It is currently in phase I clinical development in patients with solid tumors. Previously, we reported that CUDC-101 has potent antiproliferative and proapoptotic activity in cultured tumor cells and in vivo xenograft models. We now show that cancer cells that have acquired resistance to single-target EGFR inhibitors through upregulation of AXL or loss of E-cadherin remain sensitive to CUDC-101, which inhibits MET- and AXL-mediated signaling, restores E-cadherin expression, and reduces cell migration. CUDC-101 also efficiently inhibited the proliferation of MET-overexpressing non–small cell lung cancer and gastric cancer cell lines and inhibited the migration and invasion of invasive tumor cells. Taken together, these results suggest that coupling HDAC and HER2 inhibitory activities to an EGFR inhibitor may potentially be effective in overcoming drug resistance and preventing cancer cell migration. Mol Cancer Ther; 12(6); 925–36. ©2013 AACR.

Introduction

During the past several years, 4 EGF receptor (EGFR) inhibitors, gefitinib, erlotinib, cetuximab, and panitumumab, have been approved and have become standard-of-care for use in patients with cancer. However, the activity reported in unselected patients has been quite limited. In addition, those patients who achieve clinical benefit typically develop drug resistance (1–3). The extent of intrinsic and acquired resistance to EGFR inhibitors thus leaves ample room for further anticancer drug development. Several potential mechanisms of resistance to EGFR inhibitors have been identified in the last decade, including secondary EGFR mutations (4–6), expression of mutant MET (8, 9), HER2 and HER3 reactivation and overexpression-induced oncogenic shift (10), subcellular EGFR relocalization (11), ubiquitination-induced EGFR activity (12), increased VEGF production (13), overexpression of type 1 insulin-like growth factor receptor (IGF-1R; ref. 14), and most recently overexpression of AXL (15). Notably, EGFR inhibitor-resistant tumor cell lines generated in vitro have shown cross-resistance to other receptor tyrosine kinase inhibitors (16), suggesting that combination therapy or inhibitors targeting multiple oncogenic pathways may be advantageous in overcoming resistance to EGFR inhibitors.

Previously, we reported the synthesis and characterization of a novel, multifunctional small molecule, CUDC-101, which simultaneously inhibits histone deacetylase (HDAC), EGFR, and HER2 (17, 18). The multifunctional activity of CUDC-101 offers potential advantages over single-target molecules. Compared with other EGFR and HER2 inhibitors, CUDC-101 is additionally able to reduce the levels of total and phosphorylated MET through its HDAC inhibitory activity in the non–small cell lung carcinoma (NSCLC) cell line H1993 (18). Results from both preclinical studies in tumor cell lines and clinical trials have identified MET pathway activation as an important mechanism of resistance to EGFR inhibitors with MET amplification being responsible for 5% to 20% of cases of resistance in NSCLC (8, 19, 20). Activation of MET and its ligand HGF are involved in tumor cell proliferation, survival, and invasion (21). Thus, the ability of CUDC-101 to control MET levels may prove to be beneficial in controlling tumor cell proliferation, survival, and invasion.

MET-dependent tumor cell migration and invasion requires phosphoinositide 3-kinase (PI3K) pathway activity and is suppressed by the cell–cell adhesion receptor E-cadherin, which is also an important determinant of tumor progression (22, 23). Loss of E-cadherin is sufficient
CUDC-101 targets HDACs, EGFR, and HER2 with nanomolar potency. In the present study, we tested whether the HDAC and HER2 inhibitory activities of CUDC-101 could complement its EGFR inhibitory activity by suppressing drug resistance and related tumor cell motility changes in EGFR inhibitor-resistant tumor cells. We also compared its antiproliferative and antimetastatic effects with other clinically available EGFR and HDAC inhibitors by evaluating changes in migration, invasion, and epithelial–mesenchymal transition (EMT) markers. Our results indicate that CUDC-101 is able to efficiently control the proliferation and migration of erlotinib-resistant NSCLC HCC827 cells and reduce tumor cell proliferation, migration, and invasion at least partially through downregulating MET pathway activity and E-cadherin induction. Taken together, these results suggest that CUDC-101 may potentially be effective in overcoming drug resistance and preventing cancer cell migration.

Materials and Methods

Cells and reagents

CUDC-101, vorinostat, erlotinib, gefitinib, and lapatinib were synthesized as described (17). The PCR primer pairs used for detection of E-cadherin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were obtained from Applied Biosystems and Life Technologies (E-cadherin: Applied Biosystems Catalog # 4453320; GAPDH: Life Technologies Catalog # 4326317E). The real-time PCR (RT-PCR) primers used in amplification of the EGFR T790M mutation were also custom-synthesized at Applied Biosystems as previously described (34). Cancer cell lines were obtained from the American Type Culture Collection within 6 months of this study and were maintained according to the supplier’s instructions. No authentication was done by the authors. Drug-resistant HCC827 clones were generated by continuous exposure of early-passage cells (p3) to 1 μmol/L of drug for 2 months. Single clones were selected and characterized. Cells were treated with 50 ng/mL HGF (R&D Systems) or 100 ng/mL EGF (R&D Systems) in serum-free culture medium unless otherwise indicated.

Cell growth inhibition assay

Cells were plated at 5,000 cells per well in complete culturing medium in 96-well tissue culture plates for 24 hours and then incubated with indicated compounds for 3 days at concentrations of up to 20 μmol/L in medium containing 0.5% FBS. After 72 hours, cell viability was measured using the Promega Cell Titer-Glo Luminescent Cell Viability Assay. Growth inhibitory IC50s were calculated using GraphPad Prism 5 software.

Immunoblotting, immunocytochemistry, and immunohistochemistry

Immunoblotting, immunocytochemistry, and immunohistochemistry were conducted using standard procedures. Blocking solutions for immunoblotting were purchased from Li-Cor Bioscience and used with the indicated primary antibodies (Cell Signaling Technology) and IRdye 680 and 800CW secondary antibodies (Cell Signaling Technology). Bovine serum albumin (BSA)/horse serum-based blocking solutions containing the indicated primary antibodies, and Alexa 488 and Alexa 546 secondary antibodies (Cell Signaling Technology) were used for immunocytochemistry and immunohistochemistry. 4′, 6-diamidino-2-phenylindole (DAPI) was used for nuclear staining.

Wound healing cell migration assay

The wound healing cell migration assays were conducted as described previously (35–38). Briefly, MDA-MB-231, HCC827, or erlotinib-resistant cells were plated at 100,000 cells per well in complete medium in 24-well plates and allowed to form a confluent monolayer. A wound was introduced by running a P200 pipette tip evenly across the monolayer. Migration was then induced with serum-free medium containing either 50 ng/mL HGF or 100 ng/mL EGF. Calcein AM (BD Biosciences) was used to label viable cells. Fluorescence was measured with an excitation wavelength of 549 nm and an emission wavelength of 565 nm from the bottom of plates with a Biotech Synergy2 Plate Reader.

Tumor cell migration and Matrigel invasion assays

MDA-MB-231 and HT-1080 Boyden chamber migration and Matrigel invasion assays were conducted with a modified commercially available kit (BD Biosciences; ref. 39). Briefly, MDA-MB-231 cells or HT-1080 cells in logarithmic growth phase were trypsinized and suspended in serum-free medium (containing 0.04% BSA) at densities of 500,000 cells/mL or 250,000 cells/mL. FBS (10% or 5%) was added to the bottom chamber with or without test compounds to serve as a chemoattractant. After 21 hours of incubation in a 37°C incubator, cells that had migrated or invaded to the lower wells were incubated with Calcein AM (BD Biosciences) to fluorescently label viable cells. Fluorescence was measured with an
excitation wavelength of 549 nm and an emission wavelength of 565 nm from the bottom of the chamber.

**EMT Assay**
HaCaT and NBT-II cells were plated at 50,000 cells per well in low-serum (1% FBS) medium in 24-well plates for 24 to 48 hours. The cells were treated for 2 days with or without CUDC-101, erlotinib, or vorinostat. Growth factors [EGF or hepatocyte growth factor (HGF)] were added at a concentration of 20 ng/mL after one hour of drug treatment. After 48 hours, cells were fixed and treated for immunofluorescence using anti-desmosplakin (DP1 2.15; ARP) and anti-cytokeratins (18-0059; Novex) antibodies to visualize functional intercellular junctions. For EMT quantification, cells were sorted into 3 phenotype-based groups based on immunofluorescence results: "full EMT" (isolated cells), "partial EMT" (intercellular junctions involving 20%-80% of a cell’s intercellular contact area), and "no EMT" (intercellular junctions involving more than 80% of a cell’s intercellular contact area).

**Results**

**Generation of drug-resistant NSCLC HCC827 cells**
To explore the antidrug resistance activity of CUDC-101, we first attempted to generate CUDC-101–resistant cells from EGFR inhibitor-sensitive NSCLC HCC827 cells, which harbor EGFR gene amplification and Exon 19 (E19) deletion (40). As shown in Supplementary Table ST1 and Supplementary Fig. S1, the growth inhibitory IC50 of CUDC-101 (0.09 μmol/L) is very similar to the growth inhibitory IC50 of erlotinib and gefitinib (0.03 μmol/L) in the parental HCC827 cells. When HCC827 cells were treated with 1 μmol/L of these compounds, less than one percent of cells survived at the end of a 72-hour incubation. However, resistant HCC827 clones arose following treatment with 1 μmol/L erlotinib or gefitinib for an additional week, but not with 1 μmol/L of CUDC-101 (Fig. 1B), suggesting that a subset of cells intrinsically resistant to EGFR inhibitors continually survive and proliferate despite being in the presence of erlotinib and gefitinib, but not in the presence of CUDC-101.

**Erlotinib-resistant cells are sensitive to CUDC-101 treatment**
To explore the activity of CUDC-101 against EGFR inhibitor-resistant cells, we selected 6 erlotinib-tolerant/resistant (TR) single clones after 2 months of drug exposure and compared their sensitivity with CUDC-101 and EGFR inhibitors. All HCC827-TR clones were no longer sensitive to erlotinib and gefitinib with a 500-fold or greater increase in growth inhibitory IC50 relative to the parental cell line (Supplementary Table ST1). In contrast, their sensitivity to CUDC-101 did not change significantly. The fact that all HCC827-TR clones remain sensitive to CUDC-101 indicates that CUDC-101 may be able to circumvent common EGFR-inhibitor resistance mechanisms.

**Potential mechanisms of drug resistance in erlotinib-TR clones**
To identify the mechanisms of erlotinib resistance active in the HCC827 erlotinib-TR clones, we evaluated a panel of signal transduction markers related to drug resistance. As shown in Fig. 1C, all 6 resistant clones express significantly higher levels of AXL, a tyrosine kinase receptor involved in promoting tumor cell growth, migration, invasion, metastasis, and angiogenesis (41, 42). Clones TR13, TR14, TR19, and TR20 also showed reduced E-cadherin and increased N-cadherin levels (Fig. 1C). Clones TR14, TR20, and TR21 had reduced EGFR protein expression, suggesting an intrinsic heterogeneity in the parental cell line or a loss of dependence on EGFR (Fig. 1C). Very low levels of the T790M EGFR mutation were detected in clones TR13, TR19, and TR24 compared with H1975 NSCLC cells (Fig. 1D), in which the T790M mutant represents 69% of EGFR transcripts (16). This observation suggests that these clones acquired a few copies of the T790M-mutant EGFR gene while still expressing a large amount of erlotinib-sensitive E19-deleted EGFR protein.

Regardless of their EGFR expression levels, all TR clones show similar levels of AKT activation (Fig. 1C). However, this AKT activity is not due to MET amplification or IGF-1R overexpression as reported elsewhere, as no significant increase in MET and IGF-1R protein levels were detected in the TR clones (Fig. 1C). In fact, MET pathway signaling was decreased in several of the TR clones as showed by the decreased levels of phosphorylated MET in clones TR13, TR14, TR19, and TR24 (Fig. 1C).

**CUDC-101 treatment reverses drug resistance-related molecular changes in erlotinib-resistant cells**
To further understand the mechanisms by which CUDC-101 circumvents EGFR inhibitor resistance, we selected a few resistant clones and characterized their response to CUDC-101. As shown in Fig. 2A and Supplementary Fig. S2, both erlotinib and CUDC-101 inhibited activation of EGFR, MET, and AKT in the parental cells, whereas only CUDC-101 was able to prevent MET and AKT activation in the erlotinib-resistant cells. AXL levels were also significantly reduced in the HCC827 erlotinib-TR cells after treatment with CUDC-101 (Fig. 2A). Furthermore, in one of the clone expressing low levels of E-cadherin (TR19), CUDC-101 was able to restore and further increase E-cadherin mRNA and protein levels and decrease expression of the E-cadherin suppressor ZEB1 in a dose-dependent manner (Fig. 2B and C). These results indicate that CUDC-101 can counteract potential mechanisms of drug resistance in erlotinib-resistant NSCLC cells.

Because AXL, E-cadherin, and N-cadherin are known to play important roles in drug resistance and metastasis (15, 43–45), we further investigated the effects of CUDC-101 on cell migration. To this end, we showed that CUDC-101 reduced HGF-induced migration of TR19 cells in the in vitro wound-healing migration assay more effectively than erlotinib (Fig. 2D). These data...
suggest that CUDC-101 may potentially overcome erlotinib resistance by controlling both cancer cell proliferation and migration.

Cancer cells harboring MET amplification are sensitive to CUDC-101

Amplification of the MET proto-oncogene has been identified as a mechanism of resistance to EGFR inhibitors in both in vitro studies and patient samples (8). The resulting MET overexpression leads to HER3 activation independent of EGFR, allowing continued activation of downstream signaling in the presence of EGFR inhibitors (8). This improper activation of MET has been shown to bestow proliferative, survival, and invasive/metastatic properties on cancer cells (46). To investigate the effect of CUDC-101 in the presence of MET amplification, we
surveyed a panel of tumor cell lines for MET expression levels and showed that NSCLC cell lines (H1993, NCI-H441, and EBC-1) and gastric tumor cell lines (Hs746T, SNU-5, and MKN45) expressing high levels of MET are sensitive to CUDC-101 treatment at submicromolar IC50s (Fig. 3A). In contrast, the HDAC inhibitor vorinostat and the EGFR inhibitor erlotinib showed minimal or no growth inhibitory effect in most of these cell lines (Fig. 3A). Treatment with CUDC-101 significantly reduced the levels of phosphorylated and total MET protein and decreased the levels of phospho-AKT (Fig. 3B), which mediates MET-induced cell migration and invasion (21). Vorinostat slightly decreased the levels of these proteins, albeit to a lesser extent than CUDC-101, whereas erlotinib had no effect. These results indicate that tumor cells expressing high levels of MET are resistant to treatment with the EGFR inhibitor erlotinib but remain sensitive to CUDC-101 treatment.

CUDC-101 reduces tumor cell migration and invasion

To explore the antimeetastic activity of CUDC-101, we next investigated the effects of CUDC-101 on highly invasive tumor cell lines. An in vitro wound-healing migration assay of MDA-MB-231 human breast carcinoma cells showed that HGF- and EGF-induced migration

![Figure 2. CUDC-101 inhibits MET signaling, reduces AXL levels, attenuates cell migration, and restores E-cadherin expression in erlotinib-resistant HCC827 cells. A, immunoblotting of parental and erlotinib-TR (TR13 and TR21) HCC827 cells treated with 1 µmol/L of CUDC-101 or erlotinib in the presence of 50 ng/mL HGF for 24 hours. B, immunoblotting of E-cadherin and ZEB1 in TR19 cells treated with CUDC-101 at the indicated concentrations in the presence of 50 ng/mL HGF. C, TaqMan RT-PCR quantification of TR19 cells treated with CUDC-101 at the indicated concentrations in the presence of 50 ng/mL HGF. Statistical significance was determined by a standard t test (\( \cdot \cdot \cdot P < 0.01; \cdot \cdot P < 0.05 \)). D, HGF-induced (50 ng/mL) cell migration of erlotinib-TR clone TR19 treated with 1 µmol/L CUDC-101 or erlotinib in the wound-healing migration assay.](/resource/1535-7163-MCT-12-1045/12-6-Figure-2.pdf)
was significantly reduced by 1 μmol/L CUDC-101, whereas erlotinib and vorinostat were less effective even at higher concentrations (Fig. 4A and B). Using the Boyden chamber migration assay and Matrigel invasion assay, we further showed the effect of CUDC-101 on tumor cell migration and invasion in MDA-MB-231 cells and HT1080 human fibrosarcoma cells upon serum induction (Fig. 4C and D). The inhibition of migration and invasion was not a secondary effect of cell death or growth inhibition, as we detected no significant decrease in cell viability under all conditions tested (Supplementary Fig. S3).

**CUDC-101 modulates migration and invasion markers**

We next characterized the molecular mechanism of CUDC-101–mediated inhibition of tumor cell migration and invasion in MDA-MB-231 cells. As shown in Fig. 5A, 2 hours of CUDC-101 treatment decreased EGF-induced EGFR and MET phosphorylation and decreased HGF-induced EGFR phosphorylation. In addition to 24 hours of CUDC-101 treatment significantly decreased MET protein levels, likely through its HDAC inhibitory activity as we reported previously (18). At the same time, p21 protein levels were also significantly increased, suggesting that CUDC-101 induces cell-cycle arrest in these cells (Fig. 5A). At the same time, p21 protein levels were also significantly increased, suggesting that CUDC-101 induces cell-cycle arrest in these cells (Fig. 5A). MDA-MB-231 cells possess a mesenchymal phenotype and characteristically express low levels of E-cadherin and high levels of mesenchymal markers such as vimentin. Our results show that 24-hour treatment with both EGF and HGF decreases E-cadherin levels whereas 24-hour treatment with CUDC-101 restores and further increases E-cadherin levels in these cells (Fig. 5A). This CUDC-101–induced E-cadherin accumulation was also confirmed by immunofluorescence staining (Fig. 5B, left).

Simultaneously, CUDC-101 also induced morphologic changes as shown by staining for vimentin (Fig. 5B, right), a type of major intermediate filament protein that is a marker for mesenchymal cells. It has been reported that vimentin fibers exhibit a curved filamentous structure when cells are not migrating and become straight and arranged along the long axis of migrating cells (47). Our results show that upon HGF stimulation, MDA-MB-231 cells become spindle-like and the vimentin fibers straighten and reorganize along the long axis. When treated with CUDC-101 for 24 hours, the vimentin filaments become curvy again, indicative of impaired migration capacity.

Consistent with previous reports (31–33), we detected a slight increase of E-cadherin levels in response to the HDAC inhibitor vorinostat, the HER2 inhibitor lapatinib, and their combination, but not in response to erlotinib (Fig. 5C). However, CUDC-101 treatment increased E-cadherin levels with significantly greater potency than vorinostat, lapatinib, or their combination (Fig. 5C). Treatment with CUDC-101 also slightly decreased vimentin levels over time (Fig. 5A and C). E-cadherin induction was only observed after 24 hours of treatment with CUDC-101, suggesting that protein synthesis may be required. RT-PCR also showed that CUDC-101 induced E-cadherin accumulation at the transcriptional level (Fig. 5D). In agreement with these findings, CUDC-101 treatment decreased the level of ZEB1 (Fig. 5A), a zinc-finger transcriptional repressor of E-cadherin (48). We observed a similar decrease in ZEB1 levels following CUDC-101 treatment in our erlotinib-TR HCC827 clones (Fig. 2B). Taken together, these results show the ability of CUDC-101 to block cell migration at the molecular-level and suggest that CUDC-101–induced inhibition of tumor cell migration may be due to its suppression of ZEB1.
expression and the subsequent increase of E-cadherin expression through transcriptional regulation.  

**CUDC-101 inhibits EGF-induced EMT**  
Because CUDC-101 induces expression of the epithelial marker E-cadherin and reduces expression of the mesenchymal marker vimentin, we next investigated whether CUDC-101 is able to inhibit EMT in a functional assay using normal human keratinocytes (HaCaT). In the absence of growth factor treatment, HaCaT cells have a cuboidal, cobblestone morphology with tight cell–cell contacts characteristic of normal epithelial cells. Treatment with EGF leads to the loss of cell–cell contacts and the emergence of a spindle-shaped, fibroblast-like mesenchymal morphology. Treatment with CUDC-101 inhibits these EGF-induced morphologic changes and restores the epithelial phenotype, whereas vorinostat only partially inhibits EMT (Fig. 6A). To quantify the EMT phenotype, the percentage of EGF-induced partial and full cell–cell dissociation was scored and quantified. CUDC-101 completely inhibits full dissociation and decreases partial dissociation, whereas erlotinib and vorinostat are comparatively less effective (Fig. 6B). This result suggested that CUDC-101 might be able to reduce EMT.

Taken together, our results show that CUDC-101 efficiently inhibits the growth of EGFR inhibitor-resistant cells and MET-amplified cells and reduces tumor migration and invasion, suggesting that it may be a more effective clinical agent than single-agent inhibitors.

**Discussion**  
Targeted cancer therapies are promising treatment strategies, but only a subgroup of patients responds to treatment and many eventually develop drug resistance. In this study, we showed the antidrug resistance and antimetastatic activities of CUDC-101, a multitarget HDAC, EGFR, and HER2 inhibitor. Our results suggest that EGFR, HER2, and HDAC inhibition synergizes to overcome EGFR inhibitor resistance. We also provided supporting evidence for AXL overexpression and loss of E-cadherin expression as erlotinib resistance mechanisms (15, 26–28), underscoring the connection between drug resistance and metastatic processes.

In this study, AXL was overexpressed in all 6 erlotinib-TR HCC827 clones, highlighting the importance of AXL in resistance to EGFR inhibitors. AXL is a tyrosine kinase receptor expressed in various types of cancer and is involved in promoting tumor cell growth, migration, invasion, metastasis, and angiogenesis (41, 42). Overexpression of AXL has been reported as a mechanism of acquired laptatinib resistance in breast cancer cells (49), acquired imatinib resistance in gastrointestinal stromal tumors (50), and most recently erlotinib resistance in NSCLC cells and patient specimens (15). In addition, an anti-AXL monoclonal antibody YW327.6S2 has been reported to enhance the effect of erlotinib in NSCLC models (51). PI3K is a potential downstream mediator of AXL signaling (52), suggesting that AXL overexpression might be responsible for the observed activation of the PI3K pathway in our HCC827 erlotinib-TR clones.

A recent study found that AXL expression was increased (2-fold or more) in 20% of samples of patients with NSCLC following the development of resistance to the EGFR inhibitors erlotinib or gefitinib (15), indicating the importance of controlling AXL overexpression in treating EGFR inhibitor–resistant NSCLC. Although the mechanism of AXL overexpression is unknown, recent studies examining AXL overexpression-mediated EGFR inhibitor resistance in NSCLC cells found that AXL was not amplified or mutated (15), suggesting that AXL may be upregulated at the transcriptional level, perhaps by epigenetic mechanisms. Treatment of our HCC827 erlotinib-TR clones with CUDC-101 resulted in decreased levels of AXL, raising the possibility that CUDC-101–mediated HDAC inhibition may suppress AXL expression.

Several of the erlotinib-TR clones identified in this study also had reduced levels of the EGFR protein. The observation of decreased EGFR protein levels has not, to our knowledge, been reported previously as a mechanism of EGFR inhibitor resistance and is a subject for future studies. However, regardless of the baseline expression level in erlotinib-resistant cells, CUDC-101 was able to further reduce levels of total and phosphorylated EGFR and MET and downstream phosphorylated AKT in the erlotinib-TR clones where erlotinib was ineffective. As with AXL levels, the observed effect of CUDC-101 on the total protein levels of EGFR and MET is likely due to its HDAC inhibitory activity as we have shown previously (18).

Some erlotinib-resistant clones generated in this study also lost E-cadherin expression while gaining N-cadherin expression, indicative of increased tumor cell motility. We showed here that CUDC-101 could efficiently restore E-cadherin levels and control the migration of these erlotinib-resistant cells. We further showed that CUDC-101 treatment significantly decreases the motility of other invasive tumor cell lines while simultaneously increasing levels of epithelial marker and decreasing mesenchymal marker levels, indicating that CUDC-101 may potentially have antimetastatic activity.

When compared with single-target EGFR, HER2, and HDAC inhibitors, CUDC-101 more potently induced E-cadherin expression at both the protein and mRNA levels. It has been reported that both HDAC and HER2 inhibition can induce E-cadherin expression (31–33). Our data are consistent with these reports and suggest that combined HDAC and HER2 inhibitory activity may underlie the increased potency of CUDC-101 compared with single-target inhibitors. Although there is no known mechanistic link between EGFR and regulation of E-cadherin or other migration markers, E-cadherin expression has been proposed to be an important predictive marker for EGFR inhibitor sensitivity (26, 27). The ability of CUDC-101 to efficiently restore E-cadherin levels suggests that tumors
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A

EGF

HGF

0 h Control Control 0.1 µmol/L 1 µmol/L 5 µmol/L 5 µmol/L 5 µmol/L 5 µmol/L CUDC-101 Vorinostat Erlotinib

B

Wound width (% control) 0 50 100

HGF

0 h Ctrl Ctrl 0.1 µmol/L 0.1 µmol/L 0.1 µmol/L 1 µmol/L 1 µmol/L 1 µmol/L CUDC-101 Vorinostat Erlotinib

C

Wound width (% control) 0 50 100

EGF

0 h Ctrl Ctrl 0.1 µmol/L 0.1 µmol/L 0.1 µmol/L 1 µmol/L 1 µmol/L 1 µmol/L CUDC-101 Vorinostat Erlotinib

D

MDA-MB-231 Migration

MDA-MB-231 Invasion

HT1080 Migration

HT1080 Invasion

Caldesin A intensity (% control)

0 50 100 150

0 h Ctrl Ctrl 0.1 µmol/L 0.1 µmol/L 0.1 µmol/L 1 µmol/L 1 µmol/L 1 µmol/L CUDC-101 Vorinostat Erlotinib

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that are intrinsically resistant to or have acquired resistance to EGFR inhibitors may still be able to respond to CUDC-101 treatment. Mechanistically, CUDC-101 treatment decreased the level of ZEB1, a zinc-finger transcriptional repressor of E-cadherin (48), resulting in the subsequent increase of E-cadherin expression through transcriptional regulation.

MET overexpression was not observed in the erlotinib-TR HCC827 NSCLC cells generated in this study, likely due to differences in methods of generating resistant clones. To mimic clinical conditions, we chose to chronically expose the cells to 1\(\mu\)mol/L of inhibitors, instead of gradually increasing the concentration over a longer period of time as has been done previously (8). However,
we also showed that CUDC-101 could block proliferation and MET signaling in MET-amplified NSCLC cells and gastric cancer cells, indicating that CUDC-101 might also be able to efficiently control EGFR inhibitor resistance caused by MET amplification.

Initial intrinsic resistance or subsequent acquired resistance to EGFR inhibitors suggests that targeting a single node in a tumor signal transduction pathway may not effectively control tumor growth. In this study, drug-resistant cells arose from EGFR inhibitor-sensitive HCC827 cells in response to prolonged exposure to single-target EGFR inhibitors but not CUDC-101, showing the potential advantages of simultaneously targeting multiple nodes of signal transduction in cancer therapy. In addition, our findings indicate that erlotinib-TR HCC827 NSCLC cells remain sensitive to CUDC-101 treatment despite activation of different resistance mechanisms, showing the broad use of CUDC-101 in controlling EGFR-resistant cancer cells.

In summary, we have shown that the multitarget HDAC, EGFR, and HER2 inhibitor CUDC-101 could control the proliferation and migration of EGFR inhibitor-resistant NSCLC cells, and reduce tumor cell migration and invasion. Thus, as a multitarget single agent, CUDC-101 has the potential to be able to overcome drug resistance, which will be explored in future clinical studies.

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

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