ErbB-3 expression is associated with E-cadherin and their coexpression restores response to gefitinib in non-small-cell lung cancer (NSCLC)

S. E. Witta1*, R. Dziadziuszko3, K. Yoshida1, K. Hedman1, M. Varella-Garcia1, P. A. Bunn Jr1 & F. R. Hirsch1,2

1Department of Medicine–Division of Medical Oncology; 2Department of Pathology, University of Colorado Health Sciences Center and University of Colorado Cancer Center, Aurora, USA; 3Department of Oncology and Radiotherapy, Medical University of Gdansk, Gdansk, Poland

Received 13 August 2008; revised 6 October 2008; accepted 8 October 2008

Background: Epidermal growth factor receptor-(EGFR) inhibitors are effective in a subset of patients with non-small-cell lung cancer (NSCLC). We previously showed that E-cadherin expression associates with gefitinib activity. Here, we correlated the expressions of ErbB-3 and E-cadherin in NSCLC tumors and cell lines, their effect on response to gefitinib, and induction of both by the histone deacetylase (HDAC) inhibitors vorinostat and SNDX-275.

Methods: Real-time RT-PCR was carried out on RNA isolated from 91 fresh-frozen NSCLC samples and from 21 NSCLC lines. Protein expression was evaluated with western blot and flow cytometry. Apoptosis was assessed using vibrant apoptosis assay.

Results: Expressions of E-cadherin and ErbB-3 correlated significantly in primary tumors (\( r = 0.38, P < 0.001 \)) and in cell lines (\( r = 0.88, P < 0.001 \)). Cotransfection of ErbB-3 and E-cadherin in a gefitinib-resistant cell line showed enhanced apoptotic response to gefitinib. Vorinostat and SNDX-275 induced ErbB-3 and E-cadherin in gefitinib-resistant cell lines. When gefitinib-resistant lines were treated with vorinostat and gefitinib, synergistic effects were detected in four of the five lines tested.

Conclusion: ErbB-3 and E-cadherin are coexpressed and induced by HDAC inhibitors. For tumors with low ErbB-3 and E-cadherin expressions, the combination of HDAC and EGFR-tyrosine kinase inhibitors increased expression of both genes and produced more than additive apoptotic effect.

Key words: E-cadherin, EGFR, ErbB3, HDAC, NSCLC, TKI

Introduction

The epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) gefitinib and erlotinib have been studied extensively in clinical trials and produce 8%-20% response rates in patients with non-small-cell lung cancer (NSCLC) [1–3]. Ligand binding to the EGFR leads to homor heterodimerization of EGFR with other ErbB receptors including ErbB2, ErbB3, and ErbB4. EGF and other ligands stimulate ErbB3 association with phosphoinositide 3 kinase (PI3K) and activate the Akt pathway [4–6]. We and others showed that expression of ErbB3 correlates with response to EGFR-TKIs [4, 7, 8]. It is not known whether reexpression of ErbB3 influences sensitivity to EGFR-TKI.

We previously showed that EGFR-TKI sensitivity is directly related to E-cadherin expression and restoring E-cadherin expression in NSCLC cell lines by transfection augmented response to gefitinib [9].

Histone acetylases regulate the interaction of zinc finger proteins with targeted sequence in the E-cadherin promoter. Numerous histone deacetylase inhibitors (HDACi) have been identified and several are in clinical use or development. Vorinostat (suberoylanilide hydroxamic acid, SAHA, Zolinza®) was recently approved for clinical use in cutaneous T-cell lymphoma and SNDX-275 (previously MS-275) is undergoing clinical evaluation.

In our previous study, we demonstrated that sensitivity to gefitinib was enhanced with HDACi that induce E-cadherin expression [9]. The HDACi-induced sensitivity was greater than that induced by reexpressing E-cadherin by transfection. This suggests that additional proteins underlie the synergy between HDAC and EGFR-TK inhibitors in NSCLC.

Here we report the associations between ErbB3, EGFR, and E-cadherin expressions in tumors from NSCLC patients and cell lines; we examine the interaction between ErbB3 and E-cadherin and the effect of transfection of ErbB3 and
E-cadherin into a gefitinib-resistant cell line on restoring sensitivity and we evaluate the effect of vorinostat and SNDX-275 on restoring the expressions of ErbB3 and E-cadherin and sensitizing lung cancer cells to EGFR-TKIs.

**Methods**

**Patient population**
The cohort consisted of sequential 91 patients who were systematically diagnosed with stages I–III resectable NSCLC and from whom tumors were collected in tissue bank at the Medical University of Gdańsk, Poland. The majority of patients were males, with squamous cell histology, smokers, and older than 60 years (Table 1). Primary tumors were fresh frozen at the time of surgery and stored at −80°C.

**Cell culture, drugs, and MTT assay and transfection**

Cell line culture and MTT assay (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide assay) was done as previously described [7]. In brief, 21 NSCLC cell lines were used: squamous cell carcinoma (H157, HCC95, HCC15, H520), large-cell carcinoma (H460, H1299, H2126, and H1264, a derivative of H460), adenocarcinoma (Calu3, A549, H1703, H2122, H1648, HCC78, HCC193, H2009, HCC44, and H3255), and bronchioloalveolar carcinoma (H358 and H322). Six cell lines (H3255, H358, H322, Calu3, H1648, and HCC78) were very sensitive to gefitinib, with IC₅₀ values of < 1 µM in MTT growth assay. Another five cell lines (HCC15, H157, H460, H1703, and H520) were resistant with IC₅₀ values of > 10 µM. The remaining 10 cell lines had intermediate sensitivity with IC₅₀ values of 1–10 µM. The NSCLC cell lines HCC78, H2126, HCC95, H1299, HCC193, HCC44, HCC15, and H2009 were obtained from Drs John Minna and Adi Gazdar (University of Texas Southwestern Medical School, Dallas, TX). The H3255 cell line was a gift from Dr Bruce Johnson (Dana Farber Cancer Center, Boston, MA). All the other cell lines were obtained from ATCC (Rockville, MD). Gefitinib was a gift of AstraZeneca (Alderley Park, UK). Vorinostat was a gift from Merck Pharmaceuticals (Whitehouse Station, NJ). SNDX-275 (MS-275) was a gift from Schering Pharma AG (Berlin, Germany). The growth inhibitory effect was based on cultures treated with gefitinib for five consecutive days (Figure 1).

Stable hygromycin-resistant clones, H157-H3, H157-E3H3, and H157-E8H3, were generated by transfecting the ErbB3-expressing construct, pEBS7-erbB-3 (a gift from Dr M. Egeblad), into the H157 or H157-E3 and H157-E8 cell lines [10].

**RNA, primers, and quantitative real-time RT-PCR**

Total RNA was prepared from NSCLC cell lines and patients’ fresh frozen tumors using the RNAeasy kit (Qiagen). Complementary DNA (cDNA) was transcribed from 1 µg of each sample using AffinityScript™ QPCR cDNA Synthesis Kit (Stratagene, La Jolla, CA). Quantitative Real-Time PCR was carried out 1/20th of the cDNA reaction using the Brilliant® SYBR® Green QPCR Core Reagent Kit (Stratagene). Amplification data were analyzed by using GENEAMP 5700 SDS software, converted into cycle numbers at a set cycle threshold (Ct values) and quantified in relation to a standard. Human adult lung RNA (Clontech Laboratories, Inc.) was used as standard at 20, 4, 0.8, and 0.16 ng in all the experiments. To normalize for the amount of input cDNA, the quantified relative amount of the generated product was divided by the amount generated for β-actin. Cycling conditions were 50°C for 10 s and 95°C for 10 min, followed by 46 cycles at 95°C for 15 s and 60°C for 1 min. All samples were carried out in triplicates. Primers were used as E-cadherin (forward) CGG GAAATGGAGTGAGGATC, (reverse) AGGATGTTAAAGCGATGGCC [9]; ErbB3 (forward) GGGTTAGGAAAGGATGTCAAC, (reverse) GGGAGGAGGGAGTACCTTTGAG; EGFR (forward) CCACCTGGCAGATCCAGC (reverse) TCGTGTGGACAGCTCAAGAC [11], and β-actin (forward) GAGGGGGCTACAGC (reverse) TCCTTAATGTCACGCA.
immunoprecipitations and western blot analysis

Cells were disrupted in lysis buffer (10 mM Tris–HCl, pH 7.5/150 mM NaCl/0.5% IGEPAL/0.5 mM PMSF/10 μg/ml leupeptin/5 μg/ml pepstatin A/2.1 μg/ml aprotinin) on ice. After sonication, the Bradford assay was used for protein quantification. For immunoprecipitation, anti-ErbB-3 antibody (2 μg per 10-cm plate) was added to the lysate. Protein lysates (30–50 μg) were separated by gel electrophoresis on 7.5%–10% polyacrylamide and analyzed by western blot using PVDF membranes (Bio-Rad Laboratories, Inc., Richmond, CA). E-cadherin, ErbB3 and β-actin antibodies (BD Biosciences, San Jose, CA; Calbiochem; Sigma-Aldrich, #A5316, Saint Louis, MO) were used at 1 : 3000, 1 : 1000, and 1 : 5000 dilutions, respectively. Detection was by horseradish peroxidase-conjugated secondary antibodies and chemiluminescence (Amersham Biosciences, Inc.).

cell cycle analysis

NSCLC cells were plated at a density of 5 × 10^5 cells/well in six well plates. Gefitinib was added to the medium after 48 h, and the cells were incubated for another 48 h. For the sequential treatment evaluation, vorinostat was added followed by gefitinib 24 h later and cells were incubated for additional 48 h. Cell death was evaluated using the Vibrant Apoptosis Assay Kit, propidium iodide, or Annexin V-APC (V#-13243 and #A53110, respectively; Molecular Probes Inc, Eugene, OR) and analyzed using flow cytometry according to manufacturer instructions.

microarray analysis of gene expression

Following one round of in vitro transcription, 20 μg cRNA was hybridized with HG-U133 set microarrays and processed per the manufacturer’s protocol (Affymetrix, Foster City, CA). Hybridization signals and detection calls were generated in BioConductor, using the germa and ally packages as described previously [7].

FISH analysis

The ERBB3 FISH probe was prepared from the BAC clone RP11-603124, acquired from BACPAC Resource Center (Children’s Hospital Oakland Research Institute, Oakland, CA). Single-colony culture was used for amplification of the selected DNA sequences and the extracted BAC DNA was tested for the presence of ERBB3 sequences with PCR primers as follows: forward ERBB3 primer: 5’-GAGCATCAGGCTATTGCCCC-3’; reverse ERBB3 primer: 5’-CAGGACAGCCTGACCC-3’ and mapped by FISH to normal human karyotype. ERBB3 DNA was labeled by nick translation reaction with SpectrumRed-conjugated 2'-deoxyuridine 5’-triphosphate using the nick translation Kit (Abbott Molecular) according to the manufacturer’s instructions. The CEP12 (D12Z3) SpectrumGreen probe (Abbott Molecular) was used as a control for chromosome 12 aneusomy. Cell lines were cultured and harvested at the log phase after mitotic arrest with colcemid and fixed in methanol : acetic acid. Dual target FISH assays were carried out as per our standard protocol. Briefly, the slides were treated in 70% acetic acid for 10–15 s, incubated in 0.008% pepsin/0.01 M HCl at 37°C for 3–3.5 min, in 1% formaldehde for 8 min, and dehydrated in ethanol series. The probe mix was applied to the slides and the areas covered and sealed. Chromosomal and probe DNAs were codenatured for 8 min at 85°C and in situ hybridizations were allowed to occur at 37°C for 20 h. Posthybridization washes were carried out with 2x SSC (0.15 M NaCl, 0.015 M Na Citrate, pH 7.0)/0.3%NP-40 at 72°C and 2XSSC for 2 min each, followed by dehydration. Chromatin was stained with 14 ml of 4’,6-diamidino-2-phenylindole (DAPI) (0.5 mg/ml in Vectashield Mounting medium). Microscope analysis was carried out using single-interference filters sets for red (Texas red), green (FITC), blue (DAPI), and triple (blue, red, green) band pass filters. Images were captured using the CytoVision workstation (Applied Imaging).

statistical methods

The distribution of analyzed variables was not normal, as assessed by Kolmogorov–Smirnov test. We used nonparametric Spearman’s rho correlation test to analyze associations between two continuous variables. Mann–Whitney U-test and Kruskal–Wallis test was used to compare two or three groups of continuous data, respectively. The influence of ErbB3 expression on progression-free or overall survival was analyzed by univariate Cox proportional hazard model and P values from Wald’s statistic were reported. Significance level of α = 0.05 was used without correction for multiple testing. All reported P values are two sided.

results

correlation between ErbB3 and E-cadherin expressions in NSCLC cell lines and primary tumor samples

Among the 21 NSCLC cell lines, a significant correlation was detected between ErbB3 and E-cadherin expressions at the RNA level, as detected by real-time RT-PCR (r = 0.88, P < 0.001) and microarray analysis (r = 0.60, P = 0.004), and at the protein level as detected by flow cytometry (r = 0.6, P = 0.004) (Figure 1). We postulated that E-cadherin expression could correlate with ErbB3 gene copy number. We evaluated the 21 NSCLC cell lines for ErbB3 copy number by FISH. There was no correlation between ErbB3 expression by RT-PCR, microarray, or flow cytometry and gene copy number, and there was no correlation between ErbB3 copy number and E-cadherin RNA or protein expression. We also found no correlation between ErbB3 gene copy number with response to gefitinib (r = −0.22, P = 0.3) (Table 2).

The expressions of ErbB3, E-cadherin, and EGFR in tumors isolated from NSCLC patients are shown in Figure 2 and the characteristics of patients in Table 1. There was significant correlation between expressions of ErbB3 and E-cadherin, E-cadherin and EGFR, and ErbB3 and EGFR (r = 0.38, P < 0.001, Figure 2A; r = 0.29, P = 0.004, Figure 2B; r = 0.21, P = 0.041, Figure 2C; respectively; correlation coefficients indicate weak association among analyzed transcript levels).

In the cohort of 91 NSCLC patients, ErbB3 expression in primary tumors by quantitative RT-PCR analyzed as a continuous variable did not associate with gender (P = 0.756), histology (adenocarcinoma versus other types, P = 0.403), age (<60 years versus ≥60 years, P = 0.543), or pathological stage (stage I versus II versus higher, P = 0.816). ErbB3 expression did not associate with progression-free or overall survival in the univariate Cox regression model (P = 0.334 and P = 0.367, respectively).

ErbB3 interacts with E-cadherin and expression of both restores response to gefitinib

The ErbB3-negative, E-cadherin-negative, EGFR-positive, and gefitinib-resistant cell line, H157, was transfected with ErbB3 (H157-H3), E-cadherin (H157-E3, H157-E8), or both (H157-E3H3, H157-E8H3). The control cell line was transfected with GFP (H157-G) (Figure 3A). We evaluated the presence of E-cadherin in lysates immunoprecipitated with ErbB3 in H157-E3H3 and in the cell line Calu3 that endogenously coexpresses both E-cadherin and ErbB3 (Figure 3B). E-cadherin coimmunoprecipitated in both cell lines indicating that ErbB3
and E-cadherin interact. As expected, no expression of E-cadherin was detected in the control cell lines, H157-G, H157-H3, and H157-E3 (Figure 3B).

We asked whether ErbB3 has influenced the apoptotic effect of gefitinib or whether it requires the presence of E-cadherin. The effect of H157-H3 on cell survival in the presence and absence of gefitinib was evaluated. There was no increase in ratio of apoptotic (Annexin V) to viable cells in H157-H3 compared with the control cell line, H157-G, in the absence of 10 μM gefitinib (Figure 3C). A 1.5-fold increase in apoptosis was detected in the presence of 10 μM gefitinib. As described previously, a 5- and 8.5-fold increase was detected in the H157-E3 and H157-E8 cells treated with gefitinib, respectively. A 64-fold increase in apoptosis/live ratio was detected in the cell line expressing ErbB3 and E-cadherin (H157-E8H3) compared with control (Figure 3C). This indicated that expressions of both ErbB3 and E-cadherin significantly increased response to gefitinib. This apoptotic effect exceeded the effect of gefitinib on the sensitive EGFR wild-type cell line, Calu3, and similar to the apoptotic effect on the H3255 cell line containing the EGFR point mutation (L858R) (Figure 3C).

The HDAC inhibitors vorinostat and SNDX-275 induce expression of ErbB3 and E-cadherin

E-cadherin expression was restored with HDACi in lung cancer cell lines [9, 12]. We tested whether ErbB3 expression was also influenced by HDAC inhibition using vorinostat or SNDX-275.

Table 2. Expression of ErbB3 and E-cadherin in NSCLC cell lines by PCR (as % of mean normalized relative expression), microarray, FISH (erbB3, as cell mean copy number) and flow cytometry (E-cadherin)

<table>
<thead>
<tr>
<th>NSCLC Cell line</th>
<th>GEF IC50</th>
<th>ErbB3 PCR Array Flow</th>
<th>FISH erbB3</th>
<th>E-Cad PCR Array Flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>H3255a</td>
<td>0.02</td>
<td>167 221 60 7.12</td>
<td>657 455 7.9</td>
<td></td>
</tr>
<tr>
<td>H358</td>
<td>0.18</td>
<td>346 73 74 3.06</td>
<td>493 305 75.9</td>
<td></td>
</tr>
<tr>
<td>H322</td>
<td>0.25</td>
<td>196 102 88 2.32</td>
<td>313 380 18.7</td>
<td></td>
</tr>
<tr>
<td>Calu3</td>
<td>0.3</td>
<td>738 416 55.3 3.19</td>
<td>206 359 58.2</td>
<td></td>
</tr>
<tr>
<td>H1648</td>
<td>0.38</td>
<td>664 46 40 6.50</td>
<td>313 183 68.3</td>
<td></td>
</tr>
<tr>
<td>HCC78</td>
<td>0.6</td>
<td>73 552 78 4.39</td>
<td>57 238 27.4</td>
<td></td>
</tr>
<tr>
<td>H2126</td>
<td>1</td>
<td>0.6 126 14.3 3.33</td>
<td>33 256 18.8</td>
<td></td>
</tr>
<tr>
<td>HCC95</td>
<td>3</td>
<td>16 97 6.6 4.16</td>
<td>6.5 102 9.4</td>
<td></td>
</tr>
<tr>
<td>H441</td>
<td>4</td>
<td>35 153 75 5.36</td>
<td>44 60 69</td>
<td></td>
</tr>
<tr>
<td>HC193</td>
<td>4</td>
<td>0 51 35 4.15</td>
<td>0 204 42</td>
<td></td>
</tr>
<tr>
<td>Colo699</td>
<td>4.2</td>
<td>19 293 59 2.21</td>
<td>0 0 2.6</td>
<td></td>
</tr>
<tr>
<td>H2122</td>
<td>5.9</td>
<td>22 35 48 3.14</td>
<td>40 108 95.5</td>
<td></td>
</tr>
<tr>
<td>A549</td>
<td>8.2</td>
<td>0 5 0 3.18</td>
<td>6 98 4.2</td>
<td></td>
</tr>
<tr>
<td>HCC44</td>
<td>8.4</td>
<td>0 1 0 5.02</td>
<td>0 0 2.9</td>
<td></td>
</tr>
<tr>
<td>H1299</td>
<td>8.6</td>
<td>0.1 1 15 6.63</td>
<td>0 0 2.9</td>
<td></td>
</tr>
<tr>
<td>H2009</td>
<td>10</td>
<td>16 21 58 6.41</td>
<td>26 451 69</td>
<td></td>
</tr>
<tr>
<td>HCC15</td>
<td>10</td>
<td>0.64 1 17 2.39</td>
<td>0.8 12 8.62</td>
<td></td>
</tr>
<tr>
<td>H1703</td>
<td>12</td>
<td>0 1 0 2.15</td>
<td>0 0 2</td>
<td></td>
</tr>
<tr>
<td>H157</td>
<td>12.8</td>
<td>0 1 0.3 3.72</td>
<td>0 0 4.74</td>
<td></td>
</tr>
<tr>
<td>H460</td>
<td>12.9</td>
<td>0 1 0 3.02</td>
<td>0 0 3.36</td>
<td></td>
</tr>
<tr>
<td>H520</td>
<td>13.6</td>
<td>0 1 0 3.19</td>
<td>0 5 2.98</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>1537</td>
<td>738 2119 1542.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*aEGFR mutant cell line.

NSCLC, non-small-cell lung cancer.

The H157, H460, H1703, H520, and HCC15 EGFR-positive, ErbB3-negative cell lines were treated with various concentrations (0, 0.2, and 2 μM) of vorinostat and SNDX-275 and the expressions of ErbB3 and E-cadherin was evaluated by quantitative RT-PCR (Figure 4). Vorinostat increased the expression of ErbB3 by 3- to 39-fold with 0.2 μM treatment (H157: 39.5, H460: 10x, H1703: 4.4x, H520: 4.3x, and HCC15: 3x) and by 11- to 41-fold with 2 μM treatment (H157: 41.5x, H460: 19x, H1703: 14.7x, H520: 10.9x, and HCC15: 18.2x) (Figure 4A). Similarly, 24 h treatment with SNDX-275 increased the expression of ErbB3 by 5- to 17-fold.
with 0.2 μM (H157: 16.9×, H460: 8.4×, H1703: 11.3×, H520: 5×, and HCC15: 17.1×) and by 12- to 50-fold with 2 μM treatment (H157: 50.5×, H460: 16×, H1703: 16.2×, H520: 12.5×, and HCC15: 14.1×) (Figure 4B). Both vorinostat and SNDX-275 upregulated the expression of E-cadherin in those cell lines (Figure 4A and B).

**vorinostat increases response to EGFR-TKI**

We tested whether vorinostat would increase sensitivity to gefitinib in cell lines where E-cadherin and ErbB3 are upregulated by vorinostat exposure. All five NSCLC cell lines that lacked E-cadherin and ErbB3 expressions were treated separately with vorinostat or gefitinib alone or the sequential combination of vorinostat followed by gefitinib 24 h later and evaluated for the apoptosis. Increased apoptosis was detected in the four cell lines that had endogenous wild-type EGFR (H157, H460, HCC15, and H1703) and no increased apoptosis was detected in the H520 cell line that lacked EGFR expression (Figure 5).

**discussion**

Since the emergence of EGFR inhibitors as treatment of NSCLC, efforts are ongoing to identify patients who would benefit more from this therapy and to develop strategies to overcome resistance. EGFR overexpression as detected by FISH analysis and EGFR mutation are two characteristics that were shown to associate with response to EGFR inhibitors [13–15]. Epithelial to mesenchymal transition is being suggested as a tumor feature that influences response [8, 9, 16, 17]. Yauch et al. [17] showed the benefit of erlotinib given concurrently with chemotherapy in patients with increased E-cadherin expression. Another study showed a correlation between ErbB3 expression and response to gefitinib [18]. Here we show the correlation between E-cadherin and ErbB3 expressions independently correlate with response to EGFR inhibitors [4, 6–9, 17, 18]. Yauch et al. [17] showed the benefit of erlotinib given concurrently with chemotherapy in patients with increased E-cadherin expression. Another study showed a correlation between ErbB3 expression and response to gefitinib [18]. Here we show the correlation between E-cadherin and ErbB3 expressions in 21 NSCLC cell lines. We also demonstrate the significant albeit weak correlation between E-cadherin, ErbB3, and EGFR expressions in a cohort of 91 patients with resected NSCLC. We previously showed that EGFR expression, as detected at the RNA level, correlates with response to treatment with EGFR inhibitors [11]. Here we suggest that the two molecules, E-cadherin and ErbB3, are coexpressed in patients with NSCLC, expression of both correlates with EGFR RNA expression, and collectively may influence response to EGFR inhibitors.

We did not find any associations of Erbb3 expression, clinical characteristics, progression-free or overall survival of NSCLC patients. It should be noted that in this limited dataset,
analysis is clearly underpowered for detecting clinically meaningful associations.

Previous studies showed that E-cadherin interacts with EGFR trans- and intracellularly and influences the EGFR signaling pathway activation [19, 20]. Here we show that E-cadherin and ErbB3 interact in a cell line that expresses both endogenously, Calu3, or when cotransfected into the H157 cell line. Engleman et al. showed that although ErbB3 couples with PI3K in gefitinib sensitive cell lines and expression correlates with response to gefitinib, restoring ErbB3 expression did not restore sensitivity to gefitinib [4]. Consistent with these results, transfection of ErbB3 alone did not restore response to gefitinib in the H157 cell line (Figure 2C). However, when the H157 cell line was cotransfected with E-cadherin, response to gefitinib was higher than in the presence of either E-cadherin or ErbB3 alone.

Epigenetic changes play an important role in tumorigenesis, growth, apoptosis, metastasis, and drug sensitivity [21–23]. Acetylation and deacetylation of histones contribute to epigenetic regulation of gene expression and altered acetylation leads to disturbed expression of genes. The Slug/Snail family, SIP1 and ZEB1 (TF-8), are zinc finger transcriptional repressor that binds to two 5′-CACCT sequences in promoters of several genes including E-cadherin [24–26]. It is well established that E-cadherin expression is regulated by histone acetylation and deacetylation. Giving the correlative expression of ErbB3 and E-cadherin, we postulated that the two molecules are regulated through similar mechanisms. We detected restoration of ErbB3 expression in NSCLC cell lines with the use of either SNDX-275 or vorinostat. The range of vorinostat and SNDX-275 doses used in this in vitro study is comparable to levels achieved in patient’s plasma as detected in clinical trials [vorinostat, 279–685 ng/ml (1.05–2.59 M) [27]; SNDX-275, 3.6–146.9 ng/ml (0.009–0.39 M) [28–30]]. Although the level of ErbB3 reactivation is lower than what is detected with E-cadherin, these results indicate that HDACi could restore ErbB3 expression directly or indirectly. We did not detect the presence of two 5′-CACCT sequences in the ErbB3 promoter indicating that the regulation of Erbb3 expression is likely indirect. Despite, reexpressing E-cadherin and ErbB3 by transfection restores activity to gefitinib and both molecules are induced by HDACi.

We previously showed that MS-275 (SNDX-275) has synergistic effect with gefitinib in NSCLC cell lines [9]. Here, we further demonstrated that another HDACi, vorinostat, has similar synergistic apoptotic effect in four NSCLC cell lines. This further confirms the synergistic effect of HDAC and EGFR inhibitors in NSCLC. It remains to be seen in clinical studies whether this synergistic effect leads to improved outcomes of patients with NSCLC.

funding

Colorado Cancer League; Specialized Programs of Research Excellence; National Cancer Institute Cancer Center Support; the International Association for the Study of Lung Cancer/ Cancer Prevention and Research Foundation Fellowship; Merck Pharmaceuticals, USA.

references


