Preclinical Development

Overcoming Erlotinib Resistance in EGFR Mutation–Positive Non–Small Cell Lung Cancer Cells by Targeting Survivin

Kunio Okamoto1, Isamu Okamoto1, Erina Hatashita1, Kiyoko Kuwata1, Haruka Yamaguchi1, Aya Kita2, Kentaro Yamanaka2, Mayumi Ono3, and Kazuhiko Nakagawa1

Abstract
Loss of PTEN was recently shown to contribute to resistance to epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKI) in EGFR mutation–positive non–small cell lung cancer (NSCLC) through activation of the protein kinase AKT. We previously showed that downregulation of the expression of the antiapoptotic protein survivin by EGFR–TKIs contributes to EGFR–TKI-induced apoptosis in EGFR mutation–positive NSCLC cells. We have now investigated the role of survivin expression in EGFR–TKI resistance induced by PTEN loss. The EGFR–TKI erlotinib did not affect survivin expression or induce apoptosis in EGFR mutation–positive NSCLC cells with PTEN loss. Downregulation of survivin either by transfection with a specific short interfering RNA or by exposure to the small-molecule survivin suppressor YM155 reversed erlotinib resistance in such cells in vitro. Furthermore, combination therapy with YM155 and erlotinib inhibited the growth of tumors formed by EGFR mutation–positive, PTEN-deficient NSCLC cells in nude mice to a greater extent than did treatment with either drug alone. These results thus indicate that persistent activation of the AKT–survivin pathway induced by PTEN loss underlies a mechanism of resistance to erlotinib-induced apoptosis in EGFR mutation–positive NSCLC. They further suggest that the targeting of survivin has the potential to overcome EGFR–TKI resistance in EGFR mutation–positive NSCLC.

Introduction

Approximately 70% of individuals with non–small cell lung cancer (NSCLC) who harbor somatic mutations in exons of the epidermal growth factor receptor (EGFR) gene that encode the tyrosine kinase domain of the receptor experience substantial tumor regression when treated with the EGFR tyrosine kinase inhibitors (TKI) gefitinib or erlotinib (1). However, most patients, even those who show a marked response to initial treatment, develop acquired resistance to EGFR–TKIs after varying periods of time (2). To date, several major mechanisms of such acquired resistance, including secondary mutation of EGFR, amplification of MET, and overexpression of hepatocyte growth factor, have been identified, and the development of pharmaceutical agents that target these mechanisms is underway (3–7). In addition, some patients are intrinsically resistant to EGFR–TKIs, even though their tumors harbor activating mutations of EGFR (8). Further characterization of the mechanisms of EGFR–TKI resistance thus is important to provide a basis for the development of effective therapies for patients who develop such resistance.

The deletion or inactivation of the PTEN gene occurs in a variety of tumor types, including melanoma as well as lung, bladder, renal, breast, endometrial, and thyroid cancer, and there are no related proteins that can compensate for the loss of PTEN function (9). The loss of PTEN results in deregulation of AKT-dependent signaling, which plays a key role in the progression of malignant cancer (10). Recent studies have shown that PTEN loss contributes to EGFR–TKI resistance in EGFR mutation–positive lung cancer through activation of the protein kinase AKT (11, 12). We recently found that EGFR–TKIs downregulate survivin expression through inhibition of the phosphoinositide 3-kinase (PI3K)–AKT signaling pathway and that such downregulation of survivin contributed to EGFR–TKI-induced apoptosis in EGFR mutation–positive NSCLC cells (13). Survivin is a member of the inhibitor of apoptosis (IAP) family of proteins and has been shown to inhibit caspases and to prevent caspase-mediated cell death (14). Persistent survivin expression might therefore be expected to result in resistance to EGFR–TKIs in EGFR mutation–positive NSCLC cells with PTEN loss.

Authors' Affiliations: 1Department of Medical Oncology, Kinki University Faculty of Medicine, Osaka-Sayama, Osaka; 2Drug Discovery Research, Astellas Pharma Inc., Tsukuba-shi, Ibaraki; and 3Department of Pharmaceutical Oncology, Graduate School of Pharmaceutical Sciences, Kyushu University, Higashi-ku, Fukuoka, Japan

Note: Supplementary material for this article is available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).

Corresponding Author: Isamu Okamoto, Department of Medical Oncology, Kinki University Faculty of Medicine, 377-2 Ohno-higashi, Osaka-Sayama, Osaka 589-8511, Japan. Phone: 81-72-8566-0221; Fax: 81-72-360-5000; E-mail: chi-okamoto@dotd.med.kindai.ac.jp
doi: 10.1158/1535-7163.MCT-11-0638
©2011 American Association for Cancer Research.

Mol Cancer Ther; 11(1); 204–13. ©2011 AACR.
We now show that erlotinib does not affect survivin expression or induce apoptosis in EGFR mutation–positive NSCLC cells with PTEN loss. We further examined whether persistent survivin expression was associated with the sensitivity of cells to EGFR–TKIs and whether modulation of survivin expression might overcome resistance to these drugs in EGFR mutation–positive NSCLC cells with PTEN loss.

Materials and Methods

Cell culture and reagents

The human NSCLC cell lines HCC827 and H1650 were obtained from American Type Culture Collection. The NSCLC cell line PC9 was obtained as described previously (15). The NSCLC cell lines PC9/GEF1-1 and PC9/GEF2-1 were generated and characterized as described previously (12). We screened all cell lines for the presence of EGFR mutations by direct DNA sequencing of exons 18 to 21 as described previously (15) for this study. All cells were passaged for 3 months or less before the renewal from frozen, early-passage stocks obtained from the indicated sources. Cells were regularly screened for mycoplasma with the use of a MycoAlert Mycoplasma Detection Kit (Lanza). All cells were cultured under a humidified atmosphere of 5% CO2 at 37°C in RPMI 1640 medium (Sigma) supplemented with 10% FBS. Erlotinib (Supplementary Fig. S1A) was obtained from Kemprotec, and YM155 (Supplementary Fig. S1B) was obtained from Astellas Pharma.

Immunoblot analysis

Cells were washed twice with ice-cold PBS and then lysed in a solution containing 20 mmol/L Tris-HCl (pH 7.5), 150 mmol/L NaCl, 1 mmol/L EDTA, 1% Triton X-100, 2.5 mmol/L sodium pyrophosphate, 1 mmol/L phenylmethylsulfonyl fluoride, and leupeptin (1 μg/ml). The protein concentration of the cell lysates was determined with the use of the Bradford reagent (Bio-Rad) and equal amounts of protein were subjected to SDS-PAGE on a 7.5% gel. The separated proteins were transferred to a nitrocellulose membrane, which was then exposed to 5% nonfat dried milk in PBS for 1 hour at room temperature before incubation overnight at 4°C with primary antibodies. Rabbit polyclonal antibodies to human phosphorylated EGFR (pY1068), to phosphorylated or total forms of extracellular signal-regulated kinase (ERK), to XIAP, to IAP-1, to Bcl-xl, to Bcl-2, to Mcl-1, to PARP, to BIM, and to PTEN were used at 1:1,000 dilution, with the exception of those to β-actin from Sigma. Mouse monoclonal antibodies to EGFR were obtained from Invitrogen. All antibodies were used at a 1:1,000 dilution, with the exception of those to β-actin (1:200). The nitrocellulose membrane was then washed with PBS containing 0.05% Tween 20 before incubation for 1 hour at room temperature with horse-radish peroxidase–conjugated goat antibodies to rabbit (Sigma) or mouse (Santa Cruz Biotechnology) immuno-globulin G. Immune complexes were finally detected with chemiluminescence reagents (Perkin-Elmer Life Science).

Gene silencing

Cells were plated at 50% to 60% confluence in 6-well plates or 25 cm2 flasks and then incubated for 24 hours before transient transfection for 24 hours with short interfering RNAs (siRNA) mixed with the Lipofectamine reagent (Invitrogen). Small interfering RNAs specific for PTEN (5'-UGAACCUGAUCAUUAAGATT-3') or survivin (5'-GAAGCGUUUGAAGAAUA-3') mRNAs as well as a corresponding scrambled (control) siRNA were obtained from Nippon EGT.

Annexin V binding assay

The binding of Annexin V to cells was measured with the use of an Annexin V–FLUOS Staining kit (Roche). Cells were harvested by exposure to trypsin-EDTA, washed with PBS, and centrifuged at 200 x g for 5 minutes. The cell pellets were resuspended in 100 μL of Annexin V–FLUOS labeling solution, incubated for 10 to 15 minutes at 15°C to 25°C, and then analyzed for fluorescence with a flow cytometer (FACSCalibur) and Cell Quest software (Becton Dickinson).

Cell growth inhibition assay

Cells were transferred to 96-well flat-bottomed plates and cultured for 24 hours before exposure for 72 hours to various concentrations of erlotinib. Tetra Color One (5 mmol/L tetrazolium monosodium salt and 0.2 mmol/L 1-methoxy-5-methyl phenazinium methylsulfate; Seikagaku Kogyo) was then added to each well, and the cells were incubated for 3 hours at 37°C before measurement of absorbance at 490 nm with a Multiskan Spectrum instrument (Thermo Labsystems). Absorbance values were expressed as a percentage of that for untreated cells.

Growth inhibition assay in vivo

All animal studies were carried out in accordance with the Recommendations for Handling of Laboratory Animals for Biomedical Research compiled by the Committee on Safety and Ethical Handling Regulations for Laboratory Animal Experiments, Kinki University. The ethical procedures followed conformed to the guidelines of the United Kingdom Coordinating Committee on Cancer Prevention Research. Tumors cells (5 x 10^6) were injected subcutaneously into the axilla of 5- to 6-week-old female athymic nude mice (BALB/c nu/nu; CLEA Japan). Treatment was initiated when tumors in each group of 6 mice achieved an average volume of 300 to 600 mm3. Treatment groups consisted of vehicle control, erlotinib alone (10 mg/kg), YM155 alone (5 mg/kg), and erlotinib plus YM155. Erlotinib was administered by oral gavage daily for 31 days, with control animals receiving a 0.5% (w/v) aqueous solution of hydroxypropylmethylcellulose as vehicle. Continuous infusion of YM155 has been found to induce tumor regression and intratumoral survivin suppression in established human hormone-refractory...
prostate cancer, non-Hodgkin lymphoma, melanoma, and NSCLC xenografts (16–20). YM155 was thus administered over 7 consecutive days (days 1 to 7) with the use of an implanted micro-osmotic pump (Alzet model 1003D; DURECT Cupertino).

Tumor volume was determined from caliper measurements of tumor length (L) and width (W) according to the formula LW²/2. Both tumor size and body weight were measured twice per week.

**Statistical analysis**
Quantitative data are presented as means ± SE from 3 independent experiments or for 6 animals per group. The significance of differences in the percentage of Annexin V-positive cells or in tumor size was evaluated with the unpaired 2-tailed Student t test. P < 0.05 was considered statistically significant.

**Results**

Differential apoptotic responses of EGFR mutation-positive NSCLC cell lines to erlotinib
We first examined the effects of the EGFR–TKI erlotinib on apoptosis in EGFR mutation-positive NSCLC cell lines (HCC827, PC9, and H1650) by staining with Annexin V. HCC827, PC9, and H1650 cells harbor an EGFR allele with an activating mutation, whereas H1650 cells also show homozygous deletion of PTEN. Erlotinib induced a marked increase in the proportion of apoptotic cells among HCC827 and PC9 cells, whereas it was without effect in H1650 cells, despite these cells harboring an activating mutation in EGFR (Fig. 1A). Immunoblot analysis of the cleaved form of PARP confirmed that erlotinib did not induce apoptosis in H1650 cells (Fig. 1B). We and others previously showed that PI3K–AKT–survivin and mitogen-activated protein (MEK)–ERK–BIM signaling pathways play important roles in EGFR–TKI-induced apoptosis (13, 21–23). We therefore next compared the effects of erlotinib on the expression level or phosphorylation status of EGFR, AKT, ERK, PTEN, survivin, and BIM in the 3 NSCLC cell lines by immunoblot analysis. Erlotinib induced the dephosphorylation of EGFR as well as that of AKT and ERK in the erlotinib-sensitive cell lines HCC827 and PC9 (Fig. 1C). In addition, erlotinib induced downregulation of survivin expression and upregulation of BIM expression in HCC827 and PC9 cells, consistent with our previous observations (13).

**Figure 1.** Differential apoptotic responses of EGFR mutation-positive NSCLC cell lines to erlotinib. A, HCC827, PC9, or H1650 cells were incubated in complete medium with or without erlotinib (100 nmol/L) for 48 hours, after which the proportion of apoptotic cells was determined by staining with fluorescein isothiocyanate–conjugated Annexin V and propidium iodide followed by flow cytometry. Data are means ± SE from 3 independent experiments. B, cells were incubated in complete medium with or without erlotinib (100 nmol/L) for 48 hours, lysed, and subjected to immunoblot analysis with antibodies to PARP or to β-actin (loading control). Bands corresponding to the intact and cleaved (cl) forms of PARP are indicated. C, cells were incubated in complete medium containing the indicated concentrations of erlotinib for 24 hours, after which cell lysates were prepared and subjected to immunoblot analysis with antibodies to phosphorylated (p) or total forms of EGFR, AKT, or ERK or to the indicated proteins. Data in B and C are representative of 3 independent experiments.
However, whereas erlotinib induced the dephosphorylation of EGFR and ERK as well as the upregulation of BIM expression in H1650 cells, it had no effect of the levels of AKT phosphorylation or survivin expression (Fig. 1C). The expression of other IAP or Bcl family members, including XIAP, IAP-1, Bcl-2, Bcl-xl, and Mcl-1, was not substantially affected by erlotinib in any of the cell lines examined. Given that PTEN inhibits AKT activation by catalyzing the dephosphorylation of phosphatidylinositol 3,4,5-triphosphate, the product of class I PI3K action, these results suggested that the resistance of some EGFR mutation–positive cells to erlotinib-induced apoptosis may be associated with persistent activation of the AKT–survivin pathway.

**The effect of erlotinib on survivin expression correlates with that on apoptosis**

We have previously established EGFR–TKI (gefitinib)-resistant sublines (PC9/GEF1-1 and PC9/GEF2-1) of PC9 cells that harbor the activating EGFR mutation of the parental cells but which have also lost PTEN expression, with the loss of PTEN contributing to their EGFR–TKI resistance (12). Both staining with Annexin V and immunoblot analysis of PARP revealed that erlotinib did not induce a substantial level of apoptosis in PC9/GEF1-1 or PC9/GEF2-1 cells (Fig. 2A and B), consistent with our previous observations (12). To investigate whether loss of PTEN expression affects the modulation of AKT–survivin signaling by erlotinib, we examined the effects of erlotinib on the expression level or phosphorylation status of EGFR, AKT, and survivin in these cell lines by immunoblot analysis. Similar to our observations with H1650 cells, erlotinib did not affect the levels of AKT phosphorylation or survivin expression in PC9/GEF1-1 and PC9/GEF2-1 cells, whereas it still induced the dephosphorylation of EGFR and ERK as well as the upregulation of BIM expression (Fig. 2C). These findings thus suggested that persistent expression of survivin in the presence of erlotinib contributes to the resistance to this drug associated with PTEN loss in EGFR mutation–positive NSCLC cells.

**Knockdown of PTEN induces persistent survivin expression and reduces erlotinib sensitivity in EGFR mutation–positive cells**

To investigate the contribution of PTEN to survivin expression and erlotinib resistance, we examined the effects of siRNA-mediated depletion of PTEN in PC9 and HCC827 cells. Transfection of these cells with PTEN siRNA attenuated the dephosphorylation of AKT and downregulation of survivin expression, without affecting the dephosphorylation of ERK and upregulation of BIM, induced by erlotinib (Fig. 3A). Staining with Annexin V also revealed that depletion of PTEN resulted in inhibition of erlotinib-induced apoptosis (Fig. 3B). Moreover, knockdown of PTEN reduced the sensitivity of HCC827 and PC9 cells to the inhibitory effect of erlotinib on cell growth (Fig. 3C). These results suggested that PTEN loss contributes to persistent survivin expression in the presence of erlotinib and to erlotinib resistance in EGFR mutation–positive cells.
Knockdown of survivin reverses erlotinib resistance induced by loss of PTEN in EGFR mutation–positive cells

To investigate further the contribution of persistent survivin expression to erlotinib resistance associated with PTEN loss, we examined the effect of siRNA-mediated depletion of survivin on erlotinib-induced apoptosis in parental PC9 cells and the EGFR–TKI-resistant sublines PC9/GEF1-1 and PC9/GEF2-1. Whereas erlotinib induced downregulation of survivin expression only in the parental PC9 cells, transfection with survivin siRNA resulted in specific depletion of survivin in both the parental and EGFR–TKI-resistant NSCLC cells (Fig. 4A). The erlotinib-induced upregulation of BIM expression was apparent in all 3 cell lines in the absence or presence of survivin siRNA (Fig. 4A). The survivin siRNA had no significant effect on erlotinib-induced apoptosis in PC9 cells, as revealed by staining with Annexin V (Fig. 4B). In contrast, depletion of survivin by transfection with the survivin siRNA sensitized PC9/GEF1-1 and PC9/GEF2-1 cells to the proapoptotic effect of erlotinib (Fig. 4B). A second siRNA targeted to a different region of survivin mRNA yielded similar results (data not shown). These observations thus suggested that persistent expression of survivin contributes to erlotinib resistance in EGFR mutation–positive cells with loss of PTEN.

YM155 reverses erlotinib resistance induced by loss of PTEN in EGFR mutation–positive cells in vitro

We next examined the effect of the combination of erlotinib and YM155 on apoptosis in NSCLC cells with EGFR–TKI resistance due to loss of PTEN. YM155 is a small-molecule agent that specifically inhibits survivin expression in various types of cancer cell lines in vitro and in vivo (16–20). Treatment of PC9/GEF1-1 or PC9/GEF2-1 cells, or of parental PC9 cells, with YM155 resulted in downregulation of survivin expression (Fig. 5A). Erlotinib induced upregulation of BIM in all 3 cell lines in the
absence or presence of YM155 (Fig. 5A). Whereas YM155 had no significant effect on erlotinib-induced apoptosis in PC9 cells, it sensitized PC9/GEF1-1 and PC9/GEF2-1 cells to the proapoptotic effect of erlotinib (Fig. 5B). In addition, whereas YM155 did not affect the sensitivity of PC9 cells to the antiproliferative effect of erlotinib, it increased that of PC9/GEF1-1 and PC9/GEF2-1 cells to this action of erlotinib (Fig. 5C). These results thus suggested that down-regulation of survivin expression by YM155 promoted the proapoptotic and antiproliferative effects of erlotinib in EGFR mutation–positive cells with loss of PTEN.

YM155 reverses erlotinib resistance induced by loss of PTEN in EGFR mutation–positive cells in vivo

We also examined the effects of siRNA-mediated depletion of survivin and of YM155 on erlotinib-induced apoptosis in H1650 cells. Transfection with the survivin siRNA resulted in specific depletion of survivin and sensitized H1650 cells to the proapoptotic effect of erlotinib (Fig. 6A). YM155 also downregulated survivin expression and sensitized H1650 cells to the induction of apoptosis by erlotinib (Fig. 6B). In addition, YM155 increased the sensitivity of H1650 cells to the inhibitory effect of erlotinib on cell growth in vitro (Fig. 6C). Finally, to determine whether the enhancement of the proapoptotic effect of erlotinib by YM155 in EGFR–TKI-resistant cells observed in vitro might also be apparent in vivo, we injected H1650 cells into nude mice to elicit the formation of solid tumors. After tumor formation, the mice were treated with erlotinib, YM155, or both drugs. Combined treatment with erlotinib and YM155 inhibited tumor growth to a greater extent than did treatment with either drug alone (Fig. 6D and E). These data suggested that YM155 enhances the response of EGFR mutation–positive tumor cells with loss of PTEN to erlotinib both in vitro and in vivo.

Discussion

Tyrosine kinases have come to be recognized as key regulators of cancer cell proliferation and apoptosis as well as of tumor angiogenesis and they are therefore considered potential targets for anticancer therapies (24). Several strategies for targeting these kinases have
been pursued, the most successful of which has been the development of small-molecule TKIs (25–27). However, increasing evidence has shown the development of acquired resistance to these drugs, and extensive preclinical studies are ongoing to provide insight into the molecular mechanisms underlying such resistance (28). We and others have recently shown that loss of PTEN contributes to EGFR–TKI resistance in EGFR mutation–positive lung cancer through the activation of AKT (11, 12). Loss of PTEN was also found to reduce responsiveness to EGFR–TKIs in patients with recurrent glioblastoma expressing EGFR variant type III, a constitutively active mutant form of EGFR (29). In addition, restoration of PTEN expression was shown to increase the susceptibility to EGFR–TKI–induced apoptosis in EGFR mutation–positive NSCLC cells with PTEN loss (11). These observations thus support the notion that PTEN loss is associated with resistance to EGFR–TKIs in EGFR-driven tumors.

However, we have now shown that erlotinib did not induce apoptosis or downregulation of survivin expression in H1650 cells, which harbor an EGFR allele with an activating mutation and also no longer manifest PTEN expression. Similar results were obtained with EGFR–TKI-resistant PC9/GEF sublines of PC9 cells that have also lost PTEN expression and manifest persistent phosphorylation of AKT. We further found that an siRNA specific for PTEN mRNA inhibited the dephosphorylation of AKT and downregulation of survivin induced by erlotinib in EGFR mutation–positive NSCLC cells. These results are consistent with our previous observation that inhibition of the EGFR–PI3K–AKT pathway contributed to downregulation of survivin expression by EGFR–TKIs in EGFR mutation–positive NSCLC cells (13). Whereas the mechanism of survivin downregulation by EGFR–TKIs remains unclear, previous studies have suggested that the PI3K–AKT pathway regulates survivin expression through modulation of transcriptional factors (30, 31). We confirmed that erlotinib downregulated the amount of

Figure 5. YM155 reverses erlotinib resistance induced by loss of PTEN in EGFR mutation–positive NSCLC cells in vitro. A, PC9, PC9/GEF1-1, or PC9/GEF2-1 cells were incubated in complete medium with or without erlotinib (100 nmol/L), YM155 (10 nmol/L), or both drugs for 24 hours, after which cell lysates were prepared and subjected to immunoblot analysis with antibodies to the indicated proteins. Data are representative of 3 independent experiments. B, cells were incubated in complete medium with or without erlotinib (100 nmol/L), YM155 (10 nmol/L), or both drugs for 48 hours, after which the proportion of apoptotic cells was determined by staining with fluorescein isothiocyanate–conjugated Annexin V and propidium iodide followed by flow cytometry. Data are means ± SE from 3 independent experiments. *, P < 0.05 for the indicated comparisons. C, cells were cultured in complete medium containing the indicated concentrations of erlotinib with or without 10 nmol/L YM155 for 72 hours, after which cell viability was assessed. The number of viable cells is expressed as a percentage of the corresponding value for cells not exposed to erlotinib. Data are means from 3 independent experiments.
survivin mRNA in EGFR mutation–positive NSCLC cells without PTEN loss (data not shown), suggesting that survivin downregulation by erlotinib is mediated, at least in part, at the transcriptional level. These results suggest that the PI3K–AKT pathway and its downstream transcriptional factors are targeted by EGFR–TKIs in the regulation of survivin expression.

In this study, we also showed that downregulation of survivin expression either by transfection with survivin siRNA or by exposure to YM155 reversed in vitro or in vivo the resistance of EGFR mutation–positive NSCLC cells to erlotinib induced by PTEN loss. On the contrary, similar to EGFR mutation–positive cells without PTEN loss, the combination of erlotinib and YM155 did not manifest a synergistic antiproliferative effect in NSCLC cells harboring wild-type EGFR alleles (data not shown), suggesting that sensitization to EGFR–TKIs by YM155 is specific for EGFR–TKI-resistant cells with both an EGFR mutation and PTEN loss. Our observations thus show that PTEN loss activates AKT signaling and that this pathway contributes to the persistence of survivin expression in the presence of erlotinib and to EGFR–TKI resistance in EGFR mutation–positive NSCLC cells.

In addition to the PI3K–AKT–survivin pathway, we and others have previously shown that the induction of BIM expression through inhibition of the MEK–ERK pathway is important for EGFR–TKI-induced apoptosis (21–23, 32). Although erlotinib induced upregulation of the proapoptotic protein BIM in EGFR mutation–positive cells regardless of PTEN status, the proapoptotic effect of erlotinib was not observed in cells with PTEN loss. These results suggest that the PI3K–AKT–survivin signaling pathway might be more important than the MEK–ERK–BIM pathway in the regulation of survival in EGFR mutation–positive cells with loss of PTEN. However, we found that the addition of YM155 to erlotinib resulted in both the downregulation of survivin and the upregulation of BIM, leading to a greater increase in the number of apoptotic cells compared with that observed with either agent alone, in EGFR mutation–positive cells with PTEN loss. These...
results suggest that, although the relative contributions of the PI3K–AKT-survivin and MEK–ERK–BIM signaling pathways to cell survival may vary among cells, concomitant downregulation of survivin and upregulation of BIM are necessary for the induction of a substantial level of apoptosis in EGFR mutation–positive cells. Nuclear factor-kB and FAS receptor signaling were also recently shown to regulate EGFR–TKI sensitivity in EGFR mutation–positive NSCLC cells (33), suggesting that multiple mechanisms underlie EGFR–TKI resistance.

We have found that the persistent activation of AKT–survivin signaling by PTEN loss represents a mechanism of primary or acquired resistance to erlotinib in EGFR mutation–positive cells. Our results show that erlotinib downregulates survivin expression in EGFR–TKI-resistant NSCLC cells but not in EGFR–TKI-resistant NSCLC cells with PTEN loss. Although we previously showed that the expression of PTEN was reduced in tumor specimens from NSCLC patients showing acquired resistance to EGFR–TKIs (12), analysis of serial tumor samples obtained before and during treatment with these drugs will be required to determine whether survivin expression is affected by such treatment. Moreover, we found that YM155 significantly enhanced the antitumor effect of erlotinib in EGFR mutation–positive NSCLC cells with PTEN loss both in vitro and in vivo. YM155, a small-molecule inhibitor of the expression of the antiapoptotic protein survivin, is currently in clinical development as the first survivin suppressant (18, 19, 34–36). This drug was found to exhibit a favorable safety tolerability profile and moderate single-agent activity in a recent phase II trial with patients with advanced, refractory NSCLC (37). Our results now suggest that further studies of combination therapy with YM155 and erlotinib are warranted in NSCLC patients with EGFR–TKI resistance induced by PTEN loss.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

This study was supported by KAKENHI (grants-in-aid for scientific research) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

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.

Received August 17, 2011; revised November 2, 2011; accepted November 2, 2011; published OnlineFirst November 10, 2011.

References

Molecular Cancer Therapeutics

Overcoming Erlotinib Resistance in EGFR Mutation–Positive Non–Small Cell Lung Cancer Cells by Targeting Survivin

Kunio Okamoto, Isamu Okamoto, Erina Hatashita, et al.

Mol Cancer Ther 2012;11:204-213. Published OnlineFirst November 10, 2011.

Updated version
Access the most recent version of this article at:
doi:10.1158/1535-7163.MCT-11-0638

Supplementary Material
Access the most recent supplemental material at:
http://mct.aacrjournals.org/content/suppl/2011/11/04/1535-7163.MCT-11-0638.DC1

Cited articles
This article cites 37 articles, 17 of which you can access for free at:
http://mct.aacrjournals.org/content/11/1/204.full.html#ref-list-1

Citing articles
This article has been cited by 5 HighWire-hosted articles. Access the articles at:
/content/11/1/204.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.