Activation of the FGF2-FGFR1 Autocrine Pathway: A Novel Mechanism of Acquired Resistance to Gefitinib in NSCLC

Hideki Terai1, Kenzo Soejima1, Hiroyuki Yasuda1, Sohei Nakayama1, Junko Hamamoto1, Daisuke Arai1, Kota Ishioka1, Keiko Ohgino1, Shinnosuke Ikemura1, Takashi Sato1, Satoshi Yoda1, Ryosuke Satomi1, Katsuhiko Naoki2, and Tomoko Betsuyaku1

Abstract

Patients with non-small cell lung cancer (NSCLC) that harbors epidermal growth factor receptor (EGFR) mutations initially respond to EGFR-tyrosine kinase inhibitors (TKI) but eventually experience relapse. Acquired resistance to EGFR-TKIs is strongly associated with patient mortality. Thus, elucidation of the mechanism of acquired resistance to EGFR-TKIs is of great importance. In this study, gefitinib-resistant cell line models were established by long-term exposure to gefitinib using the gefitinib-sensitive lung cancer cell lines, PC9 and HCC827. Expression analyses indicated that both FGFR1 and FGF2 were increased in PC9 gefitinib-resistant (PC9 GR) cells as compared with PC9 naïve (PC9 na) cells. Importantly, proliferation of gefitinib-resistant cells was dependent on the FGF2-FGFR1 pathway. Mechanistically, inhibition of either FGF2 or FGFR1 by siRNA or FGFR inhibitor (PD173074) restored gefitinib sensitivity in PC9 GR cells. These data suggest that FGF2-FGFR1 activation through an autocrine loop is a novel mechanism of acquired resistance to EGFR-TKIs. Mol Cancer Res; 11(7); 759–67. ©2013 AACR.

Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide. Non–small cell lung carcinoma (NSCLC) accounts for about 85% of lung cancers (1). The median survival time of patients with advanced NSCLC is less than 1 year (2).

Owing to the recent advances in molecular-targeted therapy, the number of patients eligible for treatment with molecular-targeted drugs is increasing. EGF receptor (EGFR)-targeted therapy using EGFR-tyrosine kinase inhibitors (EGFR-TKI) for patients harboring EGFR mutations is one of the most accepted and well-studied therapies to date. EGFR mutations, such as exon 19 deletions or the L858R point mutation in exon 21, comprise the majority of mutations and are associated with sensitivity to EGFR-TKIs in NSCLC (3, 4). Approximately, 80% of the patients harboring these mutations respond well to EGFR-TKIs. However, almost all patients with NSCLC who initially respond well to EGFR-TKI eventually develop acquired resistance (5–7). Acquired resistance makes EGFR-targeted therapy ineffective and is strongly associated with morbidity and mortality of these patients. Substantial efforts have been made to clarify the mechanisms of acquired resistance to EGFR-TKIs. Studies over the last few years have identified 2 main EGFR-TKI resistance mechanisms. One is a second mutation in the EGFR gene itself, EGFR T790M in exon 20 of EGFR (8, 9), and the other is amplification of the MET oncogene (10–12), accounting for approximately 50% and 5% to 20% of acquired resistance to EGFR-TKIs, respectively (8–11, 13). In addition to these mechanisms, various mechanisms of acquiring resistance to EGFR-TKI have been reported, including hepatocyte growth factor (HGF)-MET pathway activation through HGF overexpression (14), epithelial–mesenchymal transition (15), EGFR amplification, and transformation to small cell lung cancer (13). However, approximately 30% of the mechanisms of acquired resistance are unknown (13, 16).

Recently, autocrine signaling of fibroblast growth factors (FGF) and their receptors (FGFR) has been shown in NSCLC cell lines (17, 18) with diverse cellular functions including proliferation, differentiation, and motility. They are also reported to be implicated in tumorigenesis as candidate “driver” oncogenes (19, 20). Numerous in vitro studies revealed frequent coexpression of specific FGFs as well as FGFR1 and FGFR2 (17, 18, 21), and several independent studies showed frequent coexpression of FGF2, FGFR1, and FGFR2 in primary NSCLC specimens (22–24). Importantly, inhibition of FGFR signaling via dominant-negative FGFR1 (21), FGFR2 neutralizing antibodies (17), FGFR-TKI (18), or antisense RNA (18, 25) blocked
proliferation of tumor growth in NSCLC. These studies suggest that the FGF–FGFR autocrine growth pathway could be an important mechanism for intrinsic resistance to EGFR-TKI in NSCLC cell lines with wild-type EGFR (18).

Until now, no report has clarified the role of FGF–FGFR pathway in acquired resistance to EGFR-TKIs in NSCLC cell lines with sensitive EGFR mutations. In this study, we propose the activation of an FGF2–FGFR1 autocrine loop as a novel mechanism of acquiring resistance to EGFR-TKIs.

**Materials and Methods**

**Cell lines**

Two human NSCLC cell lines were used, PC9 [EGFR exon 19 deletion (delE746-A750)] and HCC827 (EGFR exon 19 deletion (delE746-A750)]. PC9 was kindly gifted by S. Kobayashi (Beth Israel Deaconess Medical Center, Boston, MA; ref. 26). HCC827 was purchased from the American Type Culture Collection. All cell lines were cultured in RPMI-1640 growth medium, supplemented with 10% FBS at 37°C in a humidified 5% CO2 incubator.

**Reagents**

The cell lines were treated with the following inhibitors or ligands as a single agent or in combination at various concentrations.

Gefitinib was a gift from AstraZeneca Pharmaceuticals. PD173074 was purchased from Merck KGaA. FG2 was purchased from ReproCELL.

Total EGFR antibody (#4267), phospho-c-Met (Tyr1234/1235) antibody (#3126), total c-Met antibody (#3127), phosphop44/42 mitogen-activated protein kinase (MAPK; Tyr202/Tyr204) antibody (#3126), total p44/42 MAPK antibody (#3127), phospho-Akt (Ser473; D9E) antibody (#4060), and PARP antibody (#9532) were purchased from Cell Signaling Technologies. phospho-EGFR (Tyr1068) antibody (#44788G) was purchased from Invitrogen. Total FGFR1 antibody (sc-121) was purchased from Santa Cruz Biotechnology. β-Actin antibody produced in mouse was purchased from Sigma-Aldrich.

**Cell proliferation assay**

The MTS assay was conducted according to the manufacturer’s protocol using CellTiter 96 AQueous One Solution Assay (#G3582; Promega). Briefly, 5 × 10^4 cells were seeded per well in 96-well plates and allowed to attach for 24 hours. The cells were then treated with gefitinib or PD173074 as a single agent or in combination at variable concentrations. Control cells were treated with the same concentration of the vehicle dimethyl sulfoxide (DMSO) or PBS. Seventy-two hours after treatment with the drugs, cell growth was analyzed.

**Western blot analysis**

Cell lines were grown to 30% to 40% confluence and then stimulated with inhibitors at variable concentrations. Gefitinib was used at concentrations of 0.1 to 3.0 μmol/L, whereas PD173074 was used at 1 μmol/L. Cell lines were stimulated by these inhibitors for 4 hours. As a control, cells were treated with the vehicle DMSO. Cells were washed with cold PBS and solubilized in radioimmunoprecipitation assay (RIPA) buffer (#89900; Thermo Scientific) or cell lysis buffer (#9803; Cell Signaling Technologies). Protein concentrations were calculated by BCA protein assay (#23228, #1859078; Thermo Scientific) and equal amounts of protein were denatured and reduced with sample buffer. After boiling, aliquots of the samples were subjected to electrophoresis. The fractionated proteins were transferred to polyvinylidene difluoride (PVDF) membranes. Next, the membrane was subjected to the diluted primary antibodies followed by incubation with secondary antibodies. For the detection of proteins, the membrane was incubated with agitation in LumiGLO reagent and peroxide (#7003; Cell Signaling Technologies) and exposed to X-ray film.

**Microarray analysis**

Agilent SurePrint G3 8 × 60K human array (G4851A; Agilent Technologies) was used to monitor the expression profiles of the samples. Total RNA was prepared using the RNeasy Mini Kit (#74106; Qiagen) and labeled cRNA was prepared using standard Agilent protocols. The log2 of fold change to PC9 na were calculated for each sample by using Gene Spring GX software (Agilent Technologies). These microarray data were deposited at Gene Expression Omnibus (GEO) under dataset accession no. GSE38302.

**Quantitative RT-PCR**

Total cellular RNA was prepared from the cells by using an RNeasy Mini Kit and 0.5 mg of the RNA was then reverse-transcribed to cDNA using TaqMan Reverse Transcription Reagents (N8080234; Invitrogen). For quantitative reverse transcription PCR (qRT-PCR) analysis, we used an ABI Prism 7000 Sequence Detection System (Life Technologies). Human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used for normalization of input cDNA.

**ELISA for FGF2**

PC9 na, PC9 GR, and PC9 gr1 cells were washed with cold PBS and solubilized in RIPA buffer (#89900; Thermo Scientific) according to the manufacturer’s protocol. Lysates were assayed for FGF2 using a Quantikine human FGF basic assay kit (DFB50; R&D Systems) according to the manufacturer’s instructions.

**siRNA for FGFR1, FGFR3, and FGF2**

PC9 na, PC9 GR, and PC9 gr1 cells were transfected with a final concentration of 20 nmol/L of FGFR1 siRNA, FGFR3 siRNA, FGF2 siRNA, or negative control siRNA (FGFR1: #5164 for FGFR1 siRNA #1, #5165 for FGFR1 siRNA #2, and #5166 for FGFR1 siRNA #3; FGFR3: #5168; FGF2: #5129 for FGF2 siRNA #1; and #223531...
for FGF2 siRNA #2; Life Technologies). Silencer select and Negative control mix Silencer select (Life Technologies) were used according to the manufacturer’s instructions. For transfection into the cells, SilentFect (#170-3361; Bio-Rad) was used according to the manufacturer’s protocol. Knockdown of FGFR1, FGFR3, and FGF2 expression was confirmed using qRT-PCR. For the viability assay, cells were seeded in 6-well plates at 100,000 cells per well, 24 hours after transfection with FGFR1 siRNA #1-3, FGFR3 siRNA, FGF2 siRNA #1-2, or siRNA control.

The following day, the cells transfected with siRNAs were seeded in 96-well plates at 500 cells per well and were incubated with 6 different doses of gefitinib for an additional 72 hours. Cell viability was measured by MTS assay. As a control, cells were treated with the vehicle DMSO.

**Apoptosis assay**

PC9 na cells and PC9 GR cells were seeded in 6-well plates at 100,000 cells per well for flow-cytometric analysis using BD FACSCalibur (Becton Dickinson). Then these cells were treated with gefitinib or PD173074 as a single agent or in combination, gefitinib, was used at 0.3 μmol/L, whereas PD173074 was used at 1 μmol/L. As a control, cells were treated with the same concentration of the vehicle DMSO. We analyzed the apoptotic status of these cells by using TACS Annexin V–FITC (fluorescein isothiocyanate; R&D Systems) according to the manufacturer’s protocol.

**Results**

**Establishment of gefitinib-resistant cell lines**

To explore the novel mechanism of acquired resistance to EGFR-TKIs, PC9 and HCC827 cells were cultured in gefitinib-containing medium. The concentrations of gefitinib were gradually increased as naïve cells acquired resistance to gefitinib. After approximately 6 months of exposure, gefitinib-resistant PC9 (PC9 GR) and gefitinib-resistant HCC827 (HCC827 GR) cells were established. Gefitinib-resistant clones were also established from PC9 GR and HCC827 GR cells, namely, PC9 gr1 to PC9 gr4 and HCC827 gr1 to HCC827 gr3. Acquired resistance to gefitinib was confirmed by an MTS proliferation assay for PC9 GR, HCC827 GR, and PC9 gr clones (Fig. 1A–C).

![Figure 1](https://www.aacrjournals.org/MolCancerRes/article-figures/11/7/MCR-12-0652F1A-D.jpg)

**Figure 1.** Generating gefitinib-resistant PC9 cells by serial long-term exposure of gefitinib. A–C, PC9 na cells, PC9 GR cells (A), HCC827 na cells, HCC827 GR cells (B), and clones of PC9 GR cells (C) were cultured in medium containing 10% serum for 72 hours in the presence of various concentrations of gefitinib, after which cell viability was assessed by MTS assay. These gefitinib-resistant cells could grow at 1 μmol/L gefitinib. The number of viable cells is expressed as a percentage of the value for untreated cells. Representative data are shown. Values are expressed as mean ± SD. *: P < 0.05 by Student t test: PC9 GR versus PC9 na cells (A) and HCC827 GR versus HCC827 na cells (B) at 1 μmol/L gefitinib treatment.
The calculated IC₅₀ for gefitinib of these cell lines were 35.2 nmol/L for PC9 naïve (PC9 na), 36.1 nmol/L for HCC827 naïve (HCC827 na), and more than 1 μmol/L for resistant cells.

To determine if these cells acquired resistance to gefitinib through previously reported mechanisms (EGFR T790M mutation or MET amplification) genomic DNA from PC9 GR and PC9 gr clones or HCC827 GR and HCC827 gr clones was sequenced, and Western blotting for MET was carried out. HCC827 cells acquired resistance to gefitinib through MET amplification, whereas no amplification of MET was observed in the resistant PC9 cells (Supplementary Fig. S1 and Fig. 2). The EGFR T790M mutation was not found in any of the cells (Supplementary Fig. S2). We therefore focused on PC9 cells to investigate a novel mechanism for acquired resistance to gefitinib.

Increased expression of FGF2 and FGFR1 in PC9 GR and PC9 gr clones

To clarify the mechanism through which PC9 GR cells acquired resistance to gefitinib, a comprehensive gene expression analysis in PC9 na, PC9 GR, and PC9 gr clones, gr1 and gr3 was conducted by cDNA microarray. The expression levels of FGFR1 and FGF2 were significantly higher in gefitinib-resistant cell lines especially in PC9 GR and PC9 gr1 compared with in PC9 na (Fig. 2A and B). However, the levels of no other FGFs or FGFRs were elevated in gefitinib-resistant cell lines (Fig. 2A and B and Supplementary Fig. S3). Increase in FGFR1 and FGF2 expressions were confirmed in PC9 GR and PC9 gr1 cells by gene-specific qRT-PCR (Fig. 2C). The over-expression of FGFR1 and FGF2 proteins was confirmed by Western blotting and ELISA, respectively. The protein expressions of both FGFR1 and FGF2 were higher in

Figure 2. FGF2 and FGFR1 are highly expressed in gefitinib-resistant PC9 cells. A and B, the relative expression profiles of mRNA of FGFs by microarray analysis in PC9 GR, PC9 gr1, and PC9 gr3 cells normalized to mRNA extracted from PC9 na cells. The log₂ fold change to PC9 na were calculated for each sample by using Gene Spring GX software. The expression levels of FGFR1 and FGF2 were higher in gefitinib-resistant cell lines especially in PC9 GR and PC9 gr1 compared with PC9 na. Each group of columns represents the result from 1 microarray probe. C, the mRNA expression analyzed by qRT-PCR in PC9 na, PC9 GR, and PC9 gr1 cells. qRT-PCR confirmed the high expression levels of FGFR1 and FGF2 in PC9 GR and PC9 gr1 cells. D, cell lysates were immunoblotted to detect indicated proteins. MCF7 was used as positive control of FGFR1. E, quantities of FGF2 from the lysates of PC9 na, PC9 GR, and PC9 gr1 were measured by ELISA (R&D Systems).
Acquired Resistance to Gefitinib by FGF2–FGFR1 Autocrine Loop

Figure 3. PD173074 restored the sensitivity to gefitinib in gefitinib-resistant PC9 cells. A, effects of PD173074 on the growth of PC9 na and gefitinib-resistant PC9 cells were measured by MTS assays. These cells were cultured for 72 hours in medium containing 10% serum, various concentrations of gefitinib and PD173074, after which cell viability was assessed. As a single agent, PD173074 did not affect the survival of PC9 cells up to 1 μmol/L. Representative data are shown. B, effects of PD173074 combined with gefitinib on the growth of PC9 na cells were measured by MTS assays. C, effects of PD173074 combined with gefitinib on the growth of PC9 GR cells were assessed by MTS assays. D, effects of PD173074 combined with gefitinib on the growth of PC9 gr1 cells were assessed by MTS assays. Representative data from 3 independent experiments are shown. Values are expressed as mean ± SD. PD173074, \( P < 0.05 \) by Student’s t tests; gefitinib (1 μmol/L) alone versus combination with 1 μmol/L PD173074.

resistant PC9 cells than in the parental PC9 cells (Fig. 2D and E).

DNA quantification by quantitative PCR (qPCR) revealed that the DNA copy number of FGFR1 was not increased in PC9 GR and PC9 gr1 cells as opposed to the PC9 na cells, suggesting that the elevated expression of FGFR1 was not due to gene amplification (Supplementary Fig. S4).

Restoration of sensitivity to gefitinib by inhibition of either FGFR1 or FGF2 in resistant PC9 cells

To evaluate whether elevated expressions of FGFR1 and FGF2 were functionally important in acquiring resistance to gefitinib, MTS proliferation assays for PC9 na, PC9 GR, or PC9 gr1 cells were conducted using gefitinib and/or the FGFR inhibitor, PD173074. As a single agent treatment, PD173074 (<1 μmol/L) did not affect cell proliferation in PC9 na, PC9 GR, or PC9 gr1 cells (Fig. 3A). For PC9 na cells, combination therapy with gefitinib and PD173074 did not have any synergistic effect (Fig. 3B). However, combination therapy with gefitinib and PD173074 restored sensitivity to gefitinib in PC9 GR and PC9 gr1 cells (Fig. 3C and D). These data indicated that activation of FGFR signaling contribute to acquired resistance to gefitinib.

Because PD173074 is not a specific inhibitor of FGFR1, gene-specific knockdown of FGFR1, FGFR3 as well as FGF2 by siRNA was conducted (Supplementary Fig. S5A–S5C). Sensitivity to gefitinib was restored in PC9 GR and gr1 cells transfected with 3 independent siRNAs against FGFR1 as opposed to the cells transfected with control siRNA, as shown by an MTS proliferation assay (Fig. 4A and B). However, siRNA knockdown of FGFR3 in PC9 GR and gr1 cells did not restore sensitivity to gefitinib (Supplementary Fig. S6). On the other hand, FGF2 knockdown rescued gefitinib-induced growth inhibition in the resistant PC9 cells (Fig. 4C and D). Together with the elevated expressions of FGF2 and FGFR1 (Fig. 2), these results indicate that the resistance to gefitinib of PC9 GR and PC9 gr1 cells is mediated by the activation of an FGF2–FGFR1 autocrine loop.

Induction of apoptosis by PD173074 in combination with gefitinib in the resistant PC9 cells

We conducted fluorescence-activated cell sorting (FACS) analysis to know whether a combination of PD173074 and gefitinib can induce apoptosis in PC9 GR cells. The fraction of propidium iodide (PI) and Annexin double-positive cells was 21.2% for PC9 na cells with 0.3 μmol/L gefitinib, indicating PC9 na cells underwent apoptosis with gefitinib treatment as previously described. The fraction of PI and Annexin double-positive cells was 6.1% in PC9 GR cells with 0.3 μmol/L gefitinib, meaning PC9 GR cells are resistant to gefitinib. Although the fraction of
double-positive cells increased to 15.1% in PC9 GR cells treated with 0.3 μmol/L gefitinib in combination with 1.0 μmol/L PD173073 (Fig. 5A). PD173074 alone did not induce apoptosis in PC9 na cells and PC9 GR cells.

Western blotting revealed the presence of cleaved form of PARP in PC9 GR cells only after combined treatment with gefitinib and PD173074 (Fig. 5B).

Synergistic inhibition of the downstream targets of EGFR with gefitinib and PD173074

To further investigate how FGF2–FGFR1 pathway affects resistance to gefitinib in these cell lines, the downstream targets of EGFR signaling including the mitogen-activated protein/extracellular signal–regulated kinase–extracellular signal–regulated kinase (MEK-ERK) and phosphoinositide 3-kinase (PI3K)-AKT pathways were examined.

In PC9 na cells, the phosphorylation of EGFR, ERK, and AKT was efficiently inhibited by gefitinib alone. Also, the phosphorylation of EGFR was similarly inhibited by gefitinib alone in PC9 GR cells. This means activation of FGF2–FGFR1 axis did not affect the status of EGFR itself. On the other hand, in PC9 GR cells, the phosphorylation of ERK and AKT was less efficiently inhibited by gefitinib alone. However, the inhibition of phosphorylation of ERK and AKT was observed at lower concentrations of gefitinib combined with PD173074 (Fig. 6). Together, these findings suggest that the inhibition of the EGFR and FGFR pathways synergistically inhibit the phosphorylation of downstream of EGFR in PC9 GR cells.

Discussion

The mechanisms for acquired EGFR-TKI resistance are generally divided into 2 major categories. First, target gene alteration, such as gatekeeper mutation or amplification of EGFR, and second, activation of bypass signaling, such as activation of HGF-MET signaling and the PIK3CA mutation (8, 10, 13, 14). We have shown that the FGF2–FGFR1 autocrine loop is an alternative bypass track for acquired resistance to gefitinib in this study (Fig. 7).
FGF–FGFR signals are reported to be involved in multiple biologic processes in various types of human cancers including lung cancer. Recently, 2 independent groups reported that FGFR1 was amplified in approximately 10% to 20% of squamous cell lung cancers (27, 28). They also showed that an NSCLC cell line harboring focal amplification of FGFR1 was dependent on FGFR1 activity for cell growth. However, there are only a few reports describing the association of FGF–FGFR signaling with drug resistance. One study has suggested that FGF–FGFR pathway activation is one of the important mechanisms of “intrinsic” resistance to EGFR-TKI in NSCLC cell lines with wild-type EGFR (18). Our study proposed one of the possible mechanisms of “acquired” resistance to EGFR-TKIs in NSCLC cells harboring activating EGFR mutations. Surprisingly, overexpressions of FGF2 and its receptor FGFR1 were found in single-cell–derived PC9 gr clones, meaning that both the ligand and the receptor were derived from an identical cell, suggesting that FGF2–FGFR1 signaling was activated through an autocrine mechanism. On the other hand, it is important to note that the phosphorylation status of EGFR itself was not affected by FGF2–FGFR1 activation and completely inhibited by gefitinib. Those were also observed in the cases of gefitinib resistance induced by the activation of HGF-MET signaling and considered to be common phenomena in the bypass track activation (10, 14). Evaluating the status of EGFR phosphorylation before and after EGFR-TKI treatment may be essential to know whether the mechanism for resistance to EGFR-TKI is due to the activation of bypass track or not.

How cancer cells activate this autocrine loop is still unknown. Ware and colleagues reported on rapid acquired resistance to EGFR-TKIs in NSCLC cell lines through derepression of expressions of FGFR2 and FGFR3, but they did not elucidate the relationship between the FGF–FGFR pathway and acquired resistance to EGFR-TKI in an originally gefitinib-sensitive lung cancer cell line with EGFR mutations (29). Indeed, we also confirmed the
upregulation of FGFR2 and FGFR3 48 hours after gefitinib exposure in PC9 na cells, whereas it was not observed in PC9 GR cells chronically exposed to gefitinib for more than 6 months (Supplementary Fig. S7). Different mechanisms are required to acquire resistance through the FGFR pathway between rapid and long-term exposure to gefitinib. We examined the DNA methylation levels of FGF2 and FGFR1 gene promoters to determine whether epigenetic regulation was involved in controlling the expression levels of these genes; however, we could not find any difference in DNA methylation of the promoters of these genes (data not shown). The precise mechanism for FGF2 and FGR1 autocrine activation should be further clarified in the future.

Another limitation of this study was that we had no data for the involvement of the FGF2–FGFR1 autocrine loop activation in acquired resistance to EGFR-TKI in clinically treated patients. However, as previously reported, cell line models are powerful tools to elucidate the mechanisms of acquired resistance to EGFR-TKIs. A number of lung cancer cell lines harboring activating EGFR mutations have been established and used for elucidating the mechanism of acquired resistance to EGFR-TKIs. For example, 2 major mechanisms of acquired resistance to EGFR-TKIs, namely EGFR T790M and MET amplification, were reproduced in vitro using long-term exposure to gefitinib or erlotinib (10, 30). The activation of the FGF signaling pathway could explain, in part, some of the unsolved mechanisms for EGFR-TKI resistance in patients.

In conclusion, the data suggest that the activation of an FGF2–FGFR1 autocrine loop is an alternative mechanism for EGFR-TKI resistance. Furthermore, knockdown of FGF2 or FGFR1 by siRNAs or with PD173074 inhibited the proliferation of resistant PC9 cells and restored the sensitivity to gefitinib. These results suggest that the FGF2–FGFR1 autocrine loop could be an effective target to overcome acquired as well as intrinsic resistance to EGFR-TKIs for a subgroup of patients with NSCLC. Currently, many FGFR-TKIs or neutralizing antibodies for FGFs and FGFRs are available. We expect that FGF2–FGFR1–targeted therapy in combination with EGFR-TKIs will be an alternative way to treat patients with NSCLC resistant to EGFR-TKIs.

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

Authors’ Contributions
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): H. Terai, H. Yasuda, S. Nakayama, J. Hamamoto, K. Ogihara, S. Ikemura, R. Satomi, K. Naoki

Figure 6. Effects of the combination of gefitinib and PD173074 on cell signaling in gefitinib-resistant PC9 cells. PC9 na cells and PC9 GR cells were incubated for 4 hours in medium containing 1% serum in the absence or presence of gefitinib or PD173074 as indicated. Cell lysates were then prepared and subjected to immunoblot analysis with antibodies to phosphorylated or total forms of EGFR, ERK, or AKT. β-Actin was used for loading control. The phosphorylation of ERK was completely abrogated in the resistant PC9 cells treated with gefitinib in combination with PD173074.

Figure 7. An alternative bypass track for acquired resistance to gefitinib by FGF2–FGFR1 autocrine loop.
Acquired Resistance to Gefitinib by FGF2–FGFR1 Autocrine Loop

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): H. Terai, K. Soejima, H. Yasuda, J. Hamamoto, S. Ikemura, T. Sano, R. Satomi, K. Naoki

Writing, review, and/or revision of the manuscript: H. Terai, K. Soejima, H. Yasuda, M. Nakaiyama, J. Hamamoto, S. Ikemura, K. Naoki, T. Betsuyaku

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): K. Soejima, H. Yasuda, S. Nakaiyama, J. Hamamoto, S. Ikemura, S. Yoda, K. Naoki

Study supervision: K. Soejima, H. Yasuda, S. Ikemura, K. Naoki, T. Betsuyaku

Acknowledgments

The authors thank Ms. Miyuki Yamamoto and Ms. Mikiko Shihaya for their expert technical assistance.

References


Grant Support

This work was supported in part by Grants-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Culture, Sports, Science, and Technology of Japan to K. Soejima (Grant #22590870), H. Terai (Grant #24790822), and K. Naoki (Grant #23591311).

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 November 19, 2012; revised February 4, 2013; accepted February 18, 2013; published OnlineFirst March 27, 2013.

Published OnlineFirst March 27, 2013; DOI: 10.1158/1541-7786.MCR-12-0652

www.aacrjournals.org Mol Cancer Res; 11(7) July 2013 767

Downloaded from mcr.aacrjournals.org on June 25, 2017. © 2013 American Association for Cancer Research.
Molecular Cancer Research

Activation of the FGF2-FGFR1 Autocrine Pathway: A Novel Mechanism of Acquired Resistance to Gefitinib in NSCLC

Hideki Terai, Kenzo Soejima, Hiroyuki Yasuda, et al.


Updated version
Access the most recent version of this article at: doi:10.1158/1541-7786.MCR-12-0652

Supplementary Material
Access the most recent supplemental material at: http://mcr.aacrjournals.org/content/suppl/2013/03/27/1541-7786.MCR-12-0652.DC1

Cited articles
This article cites 30 articles, 14 of which you can access for free at: http://mcr.aacrjournals.org/content/11/7/759.full.html#ref-list-1

Citing articles
This article has been cited by 18 HighWire-hosted articles. Access the articles at: /content/11/7/759.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.