

Review Article

Somatic mutations of the EGF receptor and their signal transducers affect the efficacy of EGF receptor-specific tyrosine kinase inhibitors

Noriko Gotoh

Division of Systems Biomedical Technology, Institute of Medical Science, University of Tokyo, Tokyo, Japan.

Received April 11, 2011; accepted April 25, 2011; Epub April 26, 2011; published April 30, 2011

Abstract: Non-small cell lung cancer (NSCLC) is a major subtype of lung cancer that has been the most common and most fatal cancer worldwide. Gefitinib (Iressa™) and erlotinib (Tarceva™), specific tyrosine kinase inhibitors (TKI) for the epidermal growth factor receptor (EGFR), have been demonstrated to be effective for some NSCLC patients and are pioneering molecular-targeted drugs used in the clinic for cancer. Because many studies indicate that only some patient populations benefit from these drugs, there has been an urgent need to develop diagnostic methods to select appropriate patients for whom treatment with these drugs will be beneficial. Moreover, problems of acquired resistance after long-term treatment with the drugs have emerged. In this review, I summarize the current understanding of the EGFR-activated signal transduction pathway, which plays important roles in tumorigenesis, and of the molecular mechanisms that determine the sensitivity toward EGFR-TKI.

Keywords: HER, ErbB, Ras, PI-3 kinase, lung adenocarcinoma, acquired resistance

Introduction

Lung cancer is the leading cause of death in both men and women worldwide [1]. Lung cancer is broadly divided into non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). NSCLC, which represents approximately 85% of all lung cancers, can be further divided into squamous cell carcinoma, small cell carcinoma, adenocarcinoma, large cell carcinoma, adenosquamous carcinoma, carcinoid tumor, bronchial gland carcinomas, and others, of which adenocarcinomas are the most common. Recently, molecular-targeted drugs such as gefitinib (Iressa™) and later erlotinib (Tarceva™) tyrosine kinase inhibitor (TKI), which are specific to the EGFR tyrosine kinase, have been developed. The proven efficacy of these novel-type drugs for some patients of NSCLC raised hopes that the prognosis of NSCLC could be improved. However, clinical use showed that, although some patients responded dramatically to the drug, other patients did not respond well and eventually died because of fatal side effects, such as lung fibrosis. Female patients, never-

smokers, Asians, and lung adenocarcinoma patients are noted to have higher response rates.

In this review, I first describe the basics of signal transduction of EGFR and its family members. I then describe the recent understanding of the molecular mechanisms that determine the sensitivity and lead to acquired resistance for EGFR-TKI (small molecule inhibitors).

The basics of signal transduction of the EGFR family members

The EGFR protein family contains 4 members: EGFR (also known as ERBB1/HER1), HER2/ERBB2, HER3/ERBB3, and HER4/ERBB4 [2,3]. All the receptor members have an extracellular ligand-binding region or ectodomain, a single membrane-spanning region, and a cytoplasmic region that contains a tyrosine kinase domain. Binding of the ligand to the ectodomain initiates receptor homo- and heterodimerization and activates the cytoplasmic tyrosine kinase, stimulating the intracellular signaling pathways. There

Molecular mechanisms of the efficacy of EGF receptor-specific inhibitors

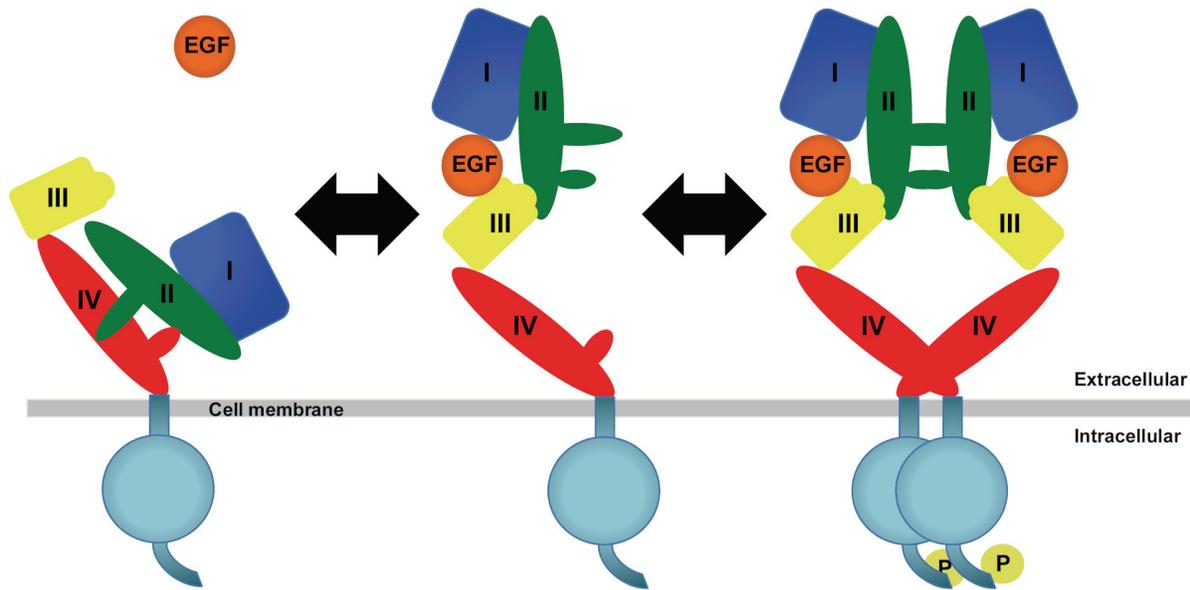


Figure 1. Model structure of the unliganded and liganded epidermal growth factor receptor (EGFR). EGFR is composed of a large extracellular ligand-binding domain, which has 4 subdomains (I–IV), followed by a transmembrane domain, a small intracellular juxtamembrane domain preceding the kinase domain, and a C-terminal tail, on which the docking sites for phosphotyrosine-binding effector molecules are found. Subdomain I and III of the extracellular domain mediate ligand binding, whereas the cysteine-rich subdomains II and IV participate in receptor dimerization. In the absence of ligand binding, the dimerization arm from subdomain II is sequestered within a monomer by interactions with subdomain IV. As shown here, ligand (EGF) binding effectively opens the receptor, releasing subdomain II from subdomain IV and exposing the dimerization arm to enable association with other receptors.

is no known ligand for HER2, and it appears that this protein can only be activated by heterodimerization with other EGFR family members. Such activation of HER2 without a ligand has been explained by its extracellular domain, which constitutively boasts a structure that is reminiscent of the ligand-bound form of HER1. This suggests that HER2 is capable of ligand-independent dimerization and is the preferred partner for the other activated HER family members. Among all the combinations of heterodimer formation, heterodimerization between HER2 and HER3 appears to be most preferentially formed and is the strongest one for signaling. HER3, on the other hand, is deficient in kinase activity because of its weak catalytic domain but can be phosphorylated by other HER family members in the heterodimeric state.

The mechanism of ligand-induced HER signaling has emerged from structural studies. As shown in **Figure 1**, the ligand-free EGFR extracellular domain displays a closed, inactive conformation that features intramolecular interactions between regions II and IV. Such a conformation

prevents both the extension of the receptor dimerization domains on subregions II and IV as well as the juxtaposition of subregions I and III to form a ligand-binding pocket. Once the ligand binds to the receptor, subregions II and IV extend away from the rest of the molecule, thus enabling receptor dimerization via intermolecular contacts involving subregions II and IV. Thus, in the open conformation, subregions I and III form a ligand-binding pocket that permits interactions between a single ligand molecule and subregions I and III. At the same time, in the cytoplasmic region of EGFR, ligand-induced dimerization is postulated to mediate kinase activation by positioning 2 cytoplasmic domains such that transphosphorylation can occur.

Activation of EGFR family members results in phosphorylation of signaling proteins to stimulate intracellular signaling pathways, which promote cell growth, proliferation, survival, differentiation, and migration (**Figure 2**). There are 2 major pathways that activate RAS via activated EGFR family members: binding to Grb2 or to Shc, both of which are adaptor proteins. In the

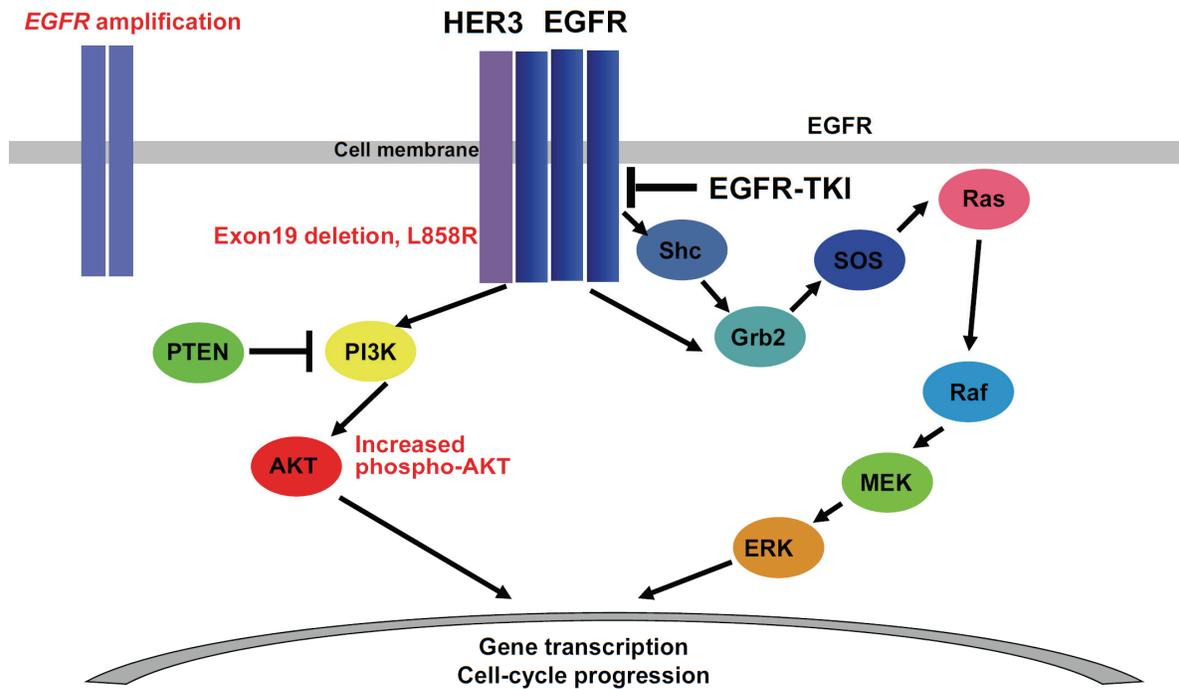


Figure 2. Schema of simplified signal transduction pathways through EGFR, and molecular mechanisms associated with sensitivity to EGFR-tyrosine kinase inhibitor (TKI).

case of EGFR, the adaptor protein Grb2 binds preferentially to the phosphorylated Y1068 of EGFR via its Src homology (SH) 2 domain. Grb2 binds to Son of Sevenless (SOS) via its SH3 domain. SOS is a GTP-exchange factor for RAS, and it increases the GTP-bound form of RAS, thereby activating RAS. The other adaptor protein, Shc, binds preferentially to the phosphorylated Y1148 of EGFR via its SH2 domain, and it undergoes tyrosine phosphorylation by the activated tyrosine kinases of the EGFR family members. Tyrosine-phosphorylated Shc binds to Grb2 through the SH2 domain of Grb2. The resulting complex between tyrosine-phosphorylated Shc and Grb2 activates RAS. The activated, GTP-bound form of RAS transiently binds to and activates RAF, a serine/threonine kinase, and phosphatidylinositol (PI)-3 kinase. Activated RAF next activates one of the major mitogen-activated protein kinase (MAPK) cascades. RAF first phosphorylates and activates MEK, and MEK subsequently phosphorylates and activates ERK. In addition to phosphorylating a number of substrates in the cytoplasm, ERK also translocates into the nucleus to phosphorylate other intranuclear substrates.

PI-3 kinase is a complex comprising a p85 subunit, adaptor proteins, and p110, a catalytic subunit. Among the EGFR family members, only tyrosine-phosphorylated HER3 directly binds to p85 via the SH2 domain of p85. Each SH2 domain of the various proteins binds to a preferred motif, and it is known that p85 preferentially binds to the pYXXM (pY, phosphorylated tyrosine; X, any amino acids; M, methionine) motif. Among the EGFR family members, only HER3 has this motif. It appears that an interaction between HER3 and the PI-3 kinase plays an important role in tumorigenesis. Although many in vitro studies have shown that activated RAS activates PI-3 kinase, this mechanism may play a minor role compared to the interaction between HER3 and PI-3 kinase. PI-3 kinase activates AKT and p70S6K/p85S6K, both of which are serine/threonine kinases.

The HER receptors are aberrantly activated in a wide range of human tumors. Increased levels of EGFR gene expression as well as overexpression of EGFR protein levels are observed in many solid tumors, including lung, head and neck, ovary, cervix, bladder, esophagus, stomach, brain, breast, endometrium, colon, and

pancreas. To circumvent this situation, a new class of drugs that specifically targets EGFR pathways has been investigated as a potential tool for cancer therapy. Antibodies directed against the extracellular domain of EGFR or against small molecule tyrosine kinase inhibitors have been developed. Gefitinib and erlotinib are small molecule compounds derived from quinazoline that compete with ATP for the ATP-binding site on EGFR to prevent autophosphorylation, with the effect of blocking signal transduction.

EGFR mutations

Cancer tissues of lung adenocarcinoma patients responding to EGFR-TKI were shown to harbor somatic mutations in *EGFR* [4]. To date, a number of somatic mutations have been identified in the *EGFR* gene in NSCLC. Most of the mutations are present in the tyrosine kinase-encoding domain (exons 18–21) of *EGFR*. The main types of mutations are as follows: point mutations at codon 719 (G719X), deletions in exon 19, insertion mutations in exon 20, and a point mutation at codon 858 in exon 21. There are over 20 variant types of deletion, such as larger deletions, deletion plus point mutation, deletion plus insertion, and so on. However, approximately 90% of the mutations are either small deletions encompassing 5 amino acids from codon 746 through 750 in exon 19, or missense mutations resulting in leucine-to-arginine switch at codon 858 (L858R). The recurrent nature of these somatic mutations implies that specific gain-of-function properties are caused by these alterations. Deletion of exon 19 and L858R mutations cause increased and sustained phosphorylation of EGFR without ligand stimulation, and activation of downstream molecules (AKT, STAT) involved in antiapoptotic pathways.

A large number of retrospective studies have confirmed the link between the clinical characteristics associated with EGFR-TKI responses and EGFR mutations. In general, about 80% of NSCLC with *EGFR* mutations respond to EGFR-TKI, whereas 10% of tumors without *EGFR* mutations do so. Two activating mutations, namely small in-frame deletion in exon 19 and substitution of leucine for arginine at amino acid 858 in exon 21 (L858R), are strikingly correlated with EGFR-TKI sensitivity. At the present time, the most common method of activating mutation

detection is by direct sequencing of the EGFR exons 18–21 from DNA isolated from tumor cells. Several studies have reported that patients with *EGFR* mutations have a significantly longer survival than those with wild-type *EGFR* when treated with EGFR-TKI [5].

EGFR mutations in cancer tissues were predominantly found in women, never-smokers, East Asians, and adenocarcinoma patients. In Japan, the frequency of *EGFR* mutation among total adenocarcinoma patients is up to 50%. This contrasts sharply with the fact that only about 10% of adenocarcinoma patients have *EGFR* mutations in Western countries. The reason why only some populations tend to have EGFR mutations is totally unknown. Recently, in Japan, the detection of EGFR mutations is often used for diagnostic purposes of adenocarcinoma patients in clinical examinations.

EGFR gene copy numbers

Patients with an amplification of *EGFR* gene in lung cancer tissues were shown to be more responsive to EGFR-TKI than patients with normal *EGFR* gene copy numbers [6]. In this study, Cappuzzo *et al.* studied the *EGFR* copy number, as determined by fluorescence *in situ* hybridization (FISH), in 100 patients treated with gefitinib, and reported that *EGFR* gene amplification is more predictive of patient survival after gefitinib treatment than *EGFR* mutations. Moreover, patients who have increased copies of *EGFR* gene show a significant survival following EGFR-TKI treatment in both Phase II and Phase III clinical trials. Also, patients with amplification or high polysomy of *EGFR* had longer median times to progression and showed an overall survival. Furthermore, most studies showed that amplification of *EGFR* was associated with somatic mutations in *EGFR*. In general, tumors with *EGFR* somatic mutations tend to also have gene amplification. It is thus likely that mutations and amplification are both important in determining EGFR-TKI sensitivity. In Western countries, detection of EGFR amplification has recently been introduced into patient diagnostics.

KRAS mutation

RAS genes, especially *KRAS*, have been implicated in the pathogenesis and prognosis of lung cancers. About 10–30% of NSCLC patients have *KRAS* mutations that are consistently associ-

ated with smoking [7]. The majority of the mutations lead to a guanine to thymine transversion in codon 12, which results in constitutive activation of the KRAS protein. NSCLC patients with KRAS mutations are associated with unfavorable prognosis.

It was reported that KRAS mutations are associated with a lack of tumor response to EGFR-TKI [8]. Further investigations confirmed that mutations of EGFR and KRAS are mutually exclusive, and that NSCLC patients with KRAS mutations have a poor sensitivity to EGFR-TKI [9-12]. This can be explained by the fact that the KRAS pathway is one of the important downstream signaling pathways of EGFR. EGFR activates many signaling pathways, including RAS, and activation of many of these pathways appears to be required for the full activity of tumorigenesis. In contrast, mutation of KRAS may activate signaling pathways leading to tumorigenesis that are stronger than those activated by EGFR. This difference in the strength of signaling is reflected by the fact that only the KRAS pathway is sufficient for full activity of tumorigenesis. It may be beneficial to determine which patients have KRAS mutations, by screening for KRAS mutation in the cancer tissues, in order to avoid them undergoing the EGFR-TKI therapy.

BRAF mutation

The occurrence of the BRAF mutation V600E has been frequently observed in various human cancers. V600E BRAF mutation stimulates MAPK signaling to induce proliferation. It was also reported that the BRAF gene mutation occurs in lung adenocarcinomas [13]. The frequency of BRAF mutations is relatively low, the same as is observed for HER2 mutations. However, it is also noteworthy that activating mutations in the EGFR, HER2, KRAS, and BRAF genes are mutually exclusive. Pratilas *et al.* indicated that the BRAF mutation in cell lines predicts resistance to EGFR-TKI and sensitivity to MEK inhibition in vitro. However, it is still unclear whether the patients with the BRAF mutation respond to EGFR-TKI.

PIK3CA mutation

Somatic mutations of PIK3CA encoding the p110 alpha catalytic subunit of PI-3 kinase have been found in various cancers. It was reported that 2 of 78 lung cancer patients had PIK3CA

mutations [14]. These 2 patients with the PIK3CA mutations, a female patient and a never-smoker, also had an EGFR mutation (L858R) and partially responded to EGFR-TKI. However, it is still unclear whether patients with the PIK3CA mutation would respond to EGFR-TKI. The role of a PIK3CA mutation as a predictive factor associated with resistance to EGFR-TKI requires further evaluation.

E-cadherin expression

EGFR interacts with the cell adhesion molecule E-cadherin, a calcium-dependent adhesion molecule. This association inhibits ligand-dependent EGFR activation and its signaling. It was reported that restoring E-cadherin expression increases sensitivity to EGFR-TKI in lung cancer cell lines [15]. It is known that expression of E-cadherin is inversely associated with the epithelial-mesenchymal transition.

Acquired resistance

In contrast to the inherent resistance to EGFR-TKI described above, it is common for patients to achieve a resistance phenotype against EGFR-TKI after varying periods. It was reported that the secondary mutation of the EGFR gene, resulting in threonine to methionine at codon 790 (T790M), was responsible for its acquired resistance [16]. In addition to the T790M mutation, it was also reported that D761Y, L747S, and an insertion in exon 20 are associated with EGFR-TKI resistance. Biochemical analysis demonstrated that the T790M mutation confers resistance to EGFR mutants that are usually sensitive to EGFR-TKI. After examining the crystal structure of the EGFR kinase domain, it was revealed that the position T790M is located in the ATP-binding pocket of the catalytic region and appears to be critical for EGFR-TKI binding. Thus, introducing the T790M mutation increases the ATP affinity of the receptors. Moreover, it was reported that the T790M mutation occurs in about 50% of patients with acquired resistance to EGFR-TKI. Therefore, The EGFR T790M mutation occurs in an analogous position to known resistance mutations to imatinib, a tyrosine kinase inhibitor specific for BCR-ABL1, KIT, and PDGFRA (T315I in ABL1, T674I in PDGFRA, and T670I in KIT). The conserved threonine residue among these different kinases, located near the kinase active site, is often referred to as the gatekeeper mutation.

Other mutations, namely those of D761Y, L747S, and insertions in exon 20, were reported to exist in lung cancer tissues with acquired resistance to EGFR-TKI.

It was reported that amplification of the *MET* gene is another mechanism to acquire resistance to EGFR-TKI [17]. *MET* is a receptor for the hepatocyte growth factor (HGF)/scatter factor, which is involved in the development of various human cancers. *MET* causes resistance by activating the HER3-dependent activation of the PI-3 kinase/AKT pathway. Moreover, it was reported that additional *MET* amplification occurs in 22% (4 of 18 specimens) of patients with an acquired resistance to EGFR-TKI.

Other mechanisms of obtaining acquired resistance to EGFR-TKI have been reported. It was reported that EGFR-TKI treatment stimulates the insulin-like growth factor 1 receptor (IGFIR) pathway and its downstream signaling via the EGFR-IGFIR heterodimer, and induces survivin expression that protects NSCLC cells from apoptosis [18]. Furthermore, Guix *et al.* demonstrated that downregulation of the IGF-binding protein 3 (IGFBP3) and IGFBP4, the negative regulators of IGF-I receptor signaling, contributes to the acquired resistance to EGFR-TKI in human cancer cell lines. Yano *et al.* recently reported that HGF, a ligand of *MET*, induces acquired resistance to EGFR-TKI in lung adenocarcinoma with *EGFR* mutations by activating *MET* and its downstream signaling. However, there are still other mechanisms of acquired resistance to EGFR-TKI that remain unclear. Further investigations to clarify the issue will be needed.

Conclusion

Thus far, the most reliable biomarkers that predict the efficacy of EGFR-TKI for NSCLC patients are the somatic mutations in *EGFR* and *EGFR* gene amplification. In fact, diagnostic methods to detect somatic mutations in *EGFR* have been developed using tissues, pleural fluid, and bronchoalveolar lavage (BAL), and these have been started to be used clinically. Although much effort has been given to understand the molecular mechanisms of the sensitivity and resistance to EGFR-TKI, these are still not fully understood. A novel idea, associated with novel technology, may be necessary to achieve a breakthrough in understanding this complex disease and to pro-

vide better treatment options for cancer patients.

Acknowledgments

This work was partially supported by Grant-in-Aid for Scientific Research on Innovative Areas from the Ministry of Education, Culture, Sports, Science and Technology of Japan; Grant-in-Aid for the Third Term Comprehensive 10-year Strategy for Cancer Control from the Ministry of Health, Labor and Welfare of Japan; and by grants from Naito Foundation and Cell Science Research Foundation.

Address correspondence to: Noriko Gotoh, Division of Systems Biomedical Technology, Institute of Medical Science, University of Tokyo, 4-6-1, Shirokanedai, Minato-ku, Tokyo 108-8639, Japan Tel.: +81 3 5559 5629; Fax: +81 3 5449 5425 E-mail: ngotoh@ims.u-tokyo.ac.jp

References

- [1] Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T and Thun MJ. Cancer statistics, 2008. *CA Cancer J Clin* 2008; 58: 71-96.
- [2] Yarden Y and Sliwkowski MX. Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol* 2001; 2: 127-137.
- [3] Citri A and Yarden Y. EGF-ERBB signalling: towards the systems level. *Nat Rev Mol Cell Biol* 2006; 7: 505-516.
- [4] Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, Herman P, Kaye FJ, Lindeman N, Boggon TJ, Naoki K, Sasaki H, Fujii Y, Eck MJ, Sellers WR, Johnson BE and Meyerson M. *EGFR* mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004; 304: 1497-1500.
- [5] Mitsudomi T, Kosaka T, Endoh H, Horio Y, Hida T, Mori S, Hatooka S, Shinoda M, Takahashi T and Yatabe Y. Mutations of the epidermal growth factor receptor gene predict prolonged survival after gefitinib treatment in patients with non-small-cell lung cancer with postoperative recurrence. *J Clin Oncol* 2005; 23: 2513-2520.
- [6] Cappuzzo F, Hirsch FR, Rossi E, Bartolini S, Ceresoli GL, Bemis L, Haney J, Witta S, Danenberg K, Domenichini I, Ludovini V, Magrini E, Gregorc V, Doglioni C, Sidoni A, Tonato M, Franklin WA, Crino L, Bunn PA, Jr. and Varella-Garcia M. Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer. *J Natl Cancer Inst* 2005; 97: 643-655.
- [7] Tam IY, Chung LP, Suen WS, Wang E, Wong MC, Ho KK, Lam WK, Chiu SW, Girard L, Minna JD, Gazdar AF and Wong MP. Distinct epidermal

Molecular mechanisms of the efficacy of EGF receptor-specific inhibitors

- growth factor receptor and KRAS mutation patterns in non-small cell lung cancer patients with different tobacco exposure and clinicopathologic features. *Clin Cancer Res* 2006; 12: 1647-1653.
- [8] Pao W, Wang TY, Riely GJ, Miller VA, Pan Q, Ladanyi M, Zakowski MF, Heelan RT, Kris MG and Varmus HE. KRAS mutations and primary resistance of lung adenocarcinomas to gefitinib or erlotinib. *PLoS Med* 2005; 2: e17.
- [9] Kosaka T, Yatabe Y, Endoh H, Kuwano H, Takahashi T and Mitsudomi T. Mutations of the epidermal growth factor receptor gene in lung cancer: biological and clinical implications. *Cancer Res* 2004; 64: 8919-8923.
- [10] Shigematsu H, Lin L, Takahashi T, Nomura M, Suzuki M, Wistuba, II, Fong KM, Lee H, Toyooka S, Shimizu N, Fujisawa T, Feng Z, Roth JA, Herz J, Minna JD and Gazdar AF. Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. *J Natl Cancer Inst* 2005; 97: 339-346.
- [11] Marchetti A, Martella C, Felicioni L, Barassi F, Salvatore S, Chella A, Campese PP, Iarussi T, Mucilli F, Mezzetti A, Cuccurullo F, Sacco R and Buttitta F. EGFR mutations in non-small-cell lung cancer: analysis of a large series of cases and development of a rapid and sensitive method for diagnostic screening with potential implications on pharmacologic treatment. *J Clin Oncol* 2005; 23: 857-865.
- [12] Eberhard DA, Johnson BE, Amler LC, Goddard AD, Heldens SL, Herbst RS, Ince WL, Janne PA, Januario T, Johnson DH, Klein P, Miller VA, Ostland MA, Ramies DA, Sebisano D, Stinson JA, Zhang YR, Seshagiri S and Hillan KJ. Mutations in the epidermal growth factor receptor and in KRAS are predictive and prognostic indicators in patients with non-small-cell lung cancer treated with chemotherapy alone and in combination with erlotinib. *J Clin Oncol* 2005; 23: 5900-5909.
- [13] Naoki K, Chen TH, Richards WG, Sugarbaker DJ and Meyerson M. Missense mutations of the BRAF gene in human lung adenocarcinoma. *Cancer Res* 2002; 62: 7001-7003.
- [14] Endoh H, Yatabe Y, Kosaka T, Kuwano H and Mitsudomi T. PTEN and PIK3CA expression is associated with prolonged survival after gefitinib treatment in EGFR-mutated lung cancer patients. *J Thorac Oncol* 2006; 1: 629-634.
- [15] Witta SE, Gemmill RM, Hirsch FR, Coldren CD, Hedman K, Ravdel L, Helfrich B, Dziadziuszko R, Chan DC, Sugita M, Chan Z, Baron A, Franklin W, Drabkin HA, Girard L, Gazdar AF, Minna JD and Bunn PA, Jr. Restoring E-cadherin expression increases sensitivity to epidermal growth factor receptor inhibitors in lung cancer cell lines. *Cancer Res* 2006; 66: 944-950.
- [16] Kobayashi S, Boggon TJ, Dayaram T, Janne PA, Kocher O, Meyerson M, Johnson BE, Eck MJ, Tenen DG and Halmos B. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2005; 352: 786-792.
- [17] Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO, Lindeman N, Gale CM, Zhao X, Christensen J, Kosaka T, Holmes AJ, Rogers AM, Cappuzzo F, Mok T, Lee C, Johnson BE, Cantley LC and Janne PA. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. *Science* 2007; 316: 1039-1043.
- [18] Morgillo F, Kim WY, Kim ES, Ciardiello F, Hong WK and Lee HY. Implication of the insulin-like growth factor-IR pathway in the resistance of non-small cell lung cancer cells to treatment with gefitinib. *Clin Cancer Res* 2007; 13: 2795-2803.