Cancer Chemother Pharmacol (2006) 58 (Suppl 1): s25–s31
DOI 10.1007/s00280-006-0312-8

The impact and role of *EGFR* gene mutation on non-small cell lung cancer

Shinichi Toyooka · Junichi Soh · Hisayuki Shigematsu · Motoi Aoe · Hiroshi Date

Published online: 9 November 2006

Abstract Mutations in the epidermal growth factor receptor (*EGFR*) gene have been reported in non-small cell lung cancer (NSCLC), especially in patients with adenocarcinomas, women, never smokers, and East Asians. These factors were recognized as predictors of gefitinib response in the Iressa Dose Evaluation in Advanced Lung Cancer study. Despite some contradictory arguments, somatic mutations in *EGFR* have been demonstrated to be a useful biomarker for predicting the clinical outcome of treatment with gefitinib or erlotinib, indicating the necessity of validated assays for clinical applications. Mutations in *EGFR* and *KRAS* are established carcinogenic mechanisms responsible for NSCLC. Recent studies have demonstrated that epigenetic alteration is another critical mechanism in lung carcinogenesis. Notably, *EGFR* and *KRAS* mutations are mutually exclusive, suggesting the presence of two independent pathways for the development of adenocarcinoma; however, the relationship between mutation and epigenetic alteration is not known. To address these issues, we examined the *EGFR* mutation status in Japanese patients with NSCLC by direct sequencing exons 18–21 of *EGFR*; the results were then correlated with clinicopathological factors and previously investigated epigenetic alterations. In this article, we mainly focus on: (1) the relationship between *EGFR* mutations and clinicopathological factors, (2) the relationship between *EGFR* mutations and response to gefitinib, (3) the development of a sensitive assay for detecting the major *EGFR* mutations, and (4) differences in the evolution of epigenetic alterations between *EGFR*- or *KRAS*-mediated tumorigenesis. The present results provide important data for translational research and will further our understanding of the molecular pathogenesis of NSCLC, leading to the establishment of molecular targeting strategies for the treatment of NSCLC.

Keywords EGFR · KRAS · NSCLC · Methylation · Gefitinib · Erlotinib

Introduction

The discovery of epidermal growth factor receptor (*EGFR*) gene mutations has opened the way to a new era in our understanding of molecular mechanisms, as well as the possibility of establishing molecular targeted therapies for non-small cell lung cancer (NSCLC). *EGFR* is a receptor tyrosine kinase that is highly expressed in epithelial tumors, including lung cancers [32]. After ligand binding, specific tyrosine residues in the intracellular domain are autophosphorylated, resulting in the initiation of an intracellular signaling cascade, and leading to a multitude of effects including cell proliferation, cell differentiation, angiogenesis, metastasis, and antiapoptosis—all of which are

This work was presented at the 21st Bristol–Myers Squibb Nagoya International Cancer Treatment Symposium, "Lung Cancer: Novel Therapy against Malfunctioning Molecules", 24–25 February 2006, Nagoya, Japan.

S. Toyooka (✉) · J. Soh · H. Shigematsu · M. Aoe · H. Date
Department of Cancer and Thoracic Surgery,
Graduate School of Medicine and Dentistry,
Okayama University, 2-5-1 Shikata-cho,
Okayama 700-8558, Japan
e-mail: s_toyooka@nigeka2.hospital.okayama-u.ac.jp

essential features of malignant tumors [11]. *EGFR* mutations are preferentially present in some subsets of patients with NSCLC, including women, never smokers, and patients with adenocarcinomas. These characteristics were identified as predictors of gefitinib response in the Iressa Survival Evaluation in Lung Cancer trial [13, 21, 24, 27, 28, 35, 39]. The introduction of vectors carrying *EGFR* mutations to cell lines also demonstrated an increased sensitivity to gefitinib, strongly supporting the view that the presence of *EGFR* mutations is a predictor of tumor responsiveness to gefitinib [24, 25, 27].

From a molecular perspective, *EGFR* and *KRAS* mutations have a mutually exclusive relationship with regard to carcinogenesis [21]. This fact implies the presence of two independent mechanisms leading to lung cancer [14, 21], particularly in the development of adenocarcinomas, since these two mutations are frequently observed in this histology [4, 35]. According to the "multi-step mechanism" hypothesis for carcinogenesis, the accumulation of molecular alterations is necessary to induce human cancers [46]. Alternatively, epigenetic alterations, including aberrant methylation, have also been established as an important mechanism in carcinogenesis [2, 47]. In colorectal carcinoma, a specific interaction of genetic and epigenetic alterations has been reported, suggesting that the activation of oncogenes by mutation and the inactivation of tumor suppressor genes (TSGs) by aberrant methylation are related to the mechanism underlying the generation of molecular diversity in cancer [44]. These facts suggest that study of both genetic and epigenetic alterations may be important for furthering our understanding of the molecular pathogenesis of lung cancer.

In this article, we review our previous studies and present some new related findings, focusing on the relationships between *EGFR* mutations, clinicopathological factors, gefitinib response, and epigenetic alterations.

Results and discussion

EGFR mutations of exon 19 deletions and L858R and clinicopathological features

We previously reported mutations in *EGFR* exons 18–21 in 120 cases of surgically resected NSCLC specimens and found that the majority of *EGFR* mutations consist of exon 19 deletions and exon 21 point-mutation (L858R) [39]. To clarify the effect of *EGFR* mutation on survival in a large-scale study, we additionally examined *EGFR* mutations in exons 19 and 21 in 311 resected NSCLC specimens. Possible correlations between the *EGFR* mutations and clinicopathological factors (age, sex, smoking status, histology, disease stage, and survival) were then examined in a total of 431 NSCLC samples. In a univariate analysis, *EGFR* mutations were more frequently identified in women, never smokers, and patients with an adenocarcinoma histology than in men, smokers, and patients with a non-adenocarcinoma histology, respectively. In a multivariate analysis, never smoking and adenocarcinoma histology were significantly associated with the presence of *EGFR* mutations. These findings are consistent with those of other reports, indicating that our cohort did not contain a selection bias.

Because the majority of the *EGFR* mutations were found in adenocarcinomas, subsequent analyses were limited to adenocarcinoma specimens. No significant association between the presence of *EGFR* mutations and disease stage was observed. Furthermore, a marginal significant difference in survival time was observed between patients with *EGFR* mutations and those without. Although the prognostic value of *EGFR* mutations remains controversial [21, 34, 35], our results indicate that the presence of *EGFR* mutations themselves appeared to be a weak predictor of prolonged survival in patients with lung adenocarcinoma when gefitinib was not used ($P = 0.088$) (Fig. 1).

Minor *EGFR* mutations and clinicopathological factors

In our previous study examining 673 cases of NSCLC, we reported that the frequency of deletions in exon 19 and L858R in exon 21 of *EGFR* were significantly

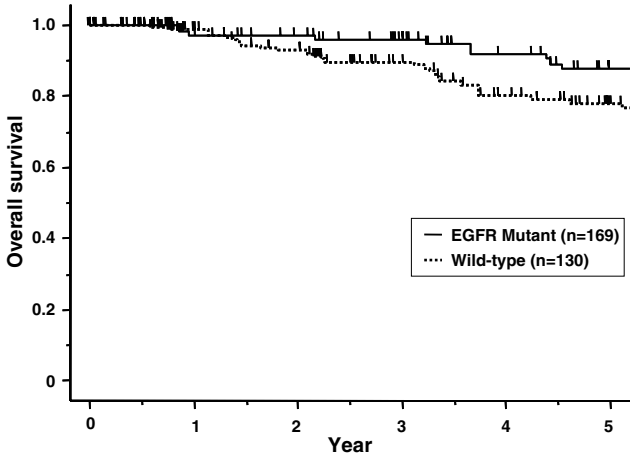

Fig. 1 Kaplan–Meier plot of survival in surgically resected lung adenocarcinoma (pathological disease stages I and II) according to *EGFR* status. Patients with *EGFR* mutation tended to show prolonged overall survival time compared to those with wild-type *EGFR* with marginal significance. $P = 0.088$

higher in women and never smokers, but that insertion mutations occurred at similar frequencies regardless of sex and smoking status [36]. Greulich et al. reported that sensitivity to gefitinib differed according to mutational types, with exon 20 insertions leading to a strong resistance to gefitinib [15]. Exon 20 insertions, exon 18 mutations, and all others mutations (except for exon 19 deletions and L858R) are regarded as minor mutations, accounting for approximately 10% of all *EGFR* mutations [35]. Despite the fact that the low incidence of these mutations makes study difficult, the effects of these minor mutations on responsiveness to EGFR-tyrosine kinase inhibitors (TKIs) should be investigated to establish individualized therapy for patients with NSCLC.

EGFR mutations and tumor responsiveness to gefitinib

EGFR mutations have been associated with tumor sensitivity to gefitinib, a finding that has aroused much interest. While numerous studies have confirmed this finding, other contentious results have also been reported; Cappuzzo et al. showed that the *EGFR* copy number status, rather than the presence of *EGFR* mutations, was significantly associated with the clinical outcome of gefitinib therapy [7]. Tsao et al. reported that neither the presence of *EGFR* mutations nor the *EGFR* copy number was related to clinical outcome in patients treated with erlotinib [45]. In our study of 21 cases that were treated with gefitinib, drug responsiveness was significantly higher among patients with tumors carrying *EGFR* mutations [39]. While previous studies from East Asia showed that the *EGFR* mutation was a predictive factor for a favorable clinical outcome, studies from North America and Europe reported controversial results on the effect of *EGFR* mutations and amplification on the clinical outcome in patients treated with EGFR-TKIs [3, 7, 10, 16, 25, 31, 37–39, 45], implying that ethnic or geographical differences in the effects of EGFR-TKIs may exist. Of note, inter-ethnic differences in polymorphisms in the *EGFR* promoter (-216G/T and -191C/A) and intron 1 (the length of a CA dinucleotide repeat) that influence *EGFR* expression level have been reported, possibly explaining not only the benefits, but also the adverse effects of EGFR-TKIs [5, 6, 22, 23]. Thus, the genotyping of these polymorphisms may be important in understanding inter-ethnic differences in *EGFR* somatic alterations, including mutations and copy number, and gefitinib sensitivity in patients with NSCLC.

Another important issue concerning *EGFR* mutations is the T790M mutation that is sometimes acquired after gefitinib treatment and that causes sensitive tumors to become resistant to gefitinib [20, 29]. We and others have reported on patients with NSCLC carrying inherent T790M and L858R mutations who did not receive any pre-treatment and who were resistant to gefitinib [37, 41]. As mentioned above, a recent finding has also demonstrated that insertions in exon 20 cause the resistance of tumors to EGFR-TKIs [15]. These findings also suggest that *EGFR* mutations may be responsible for sensitivity to EGFR-TKIs.

Highly sensitive assay for the detection of major *EGFR* mutation

We considered that the benefit of gefitinib was maximized when patients with an exon 19 deletion or a L858R mutation were selected for treatment. In clinical practice, *EGFR* mutations have to be detected in various clinical samples including not only resected specimens, but also biopsies and pleural effusions that are likely to contain plenty of non-malignant cells. For that purpose, a highly sensitive assay for detecting mutations is necessary. Several sensitive assays to detect *EGFR* mutations have been reported [17, 26]. We have developed a mutant-enriched PCR assay for the detection of exon 19 deletion and L858R [1]. Mutant-enriched PCR is a two-step PCR with intermittent digestion to eliminate wild-type alleles selectively, thus enriching the mutated alleles [19]. The sensitivity of this assay is such that, using serially diluted plasmid DNAs, it successfully detected one mutant in the presence of 2×10^3 wild-type alleles [1]. The advantage of the enriched assay was demonstrated in detecting mutations in surgically resected specimens, CT-guided and transbronchial lung biopsies (TBLB), and pleural fluid. Figure 2a shows the microscopic view of a surgically resected specimen from a patient who had received chemo-radiation therapy in the neo-adjuvant setting because of N2 disease. While direct DNA sequencing revealed no *EGFR* mutation in the resected specimen, the enriched PCR assay demonstrated the presence of L858R. The pathological findings of the resected specimen revealed few variable cancer cells with non-malignant cells, which may explain why the direct sequencing did not detect the mutation, but the mutant-enriched PCR was able to detect it from a small fraction of tumor cells. The pathological examination of TBLB and a mediastinal lymph node specimen (Fig. 2b) of this case revealed a central type of squamous cell carcinoma in a female never smoker. Direct sequencing of the lymph node specimen also revealed the L858R alteration. While *EGFR* mutations are frequently found in adenocarcinomas, we previously reported the presence of *EGFR*

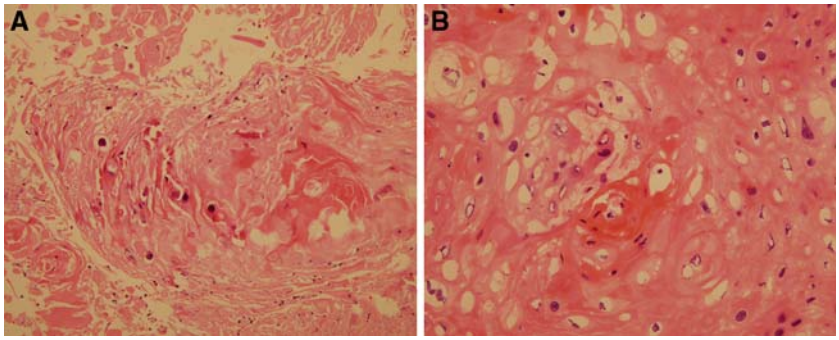

Fig. 2 Microscopic findings. **a** Resected specimen after neo-adjuvant chemo-radiotherapy. There were few variable cells in the specimen. **b** Metastatic mediastinal lymph node of the same case biopsied by mediastenoscopy before chemo-radiotherapy. The pathological finding revealed squamous cell carcinoma cells

mutations in both adenocarcinomatous and squamous cell carcinomatous components of adenosquamous carcinomas [43]. These facts suggested that *EGFR* mutation is not a crucial factor for the adenocarcinomatous phenotype of tumors.

Relationship between somatic genetic and epigenetic alterations

The aberrant methylation of TSGs is an important mechanism in human carcinogenesis [2]. We previously reported that the frequent methylation of several genes involved in lung cancer as well as the methylation of $p16^{INK4a}$, *RASSF1A*, and *APC* were closely related to smoking status, similar to the *KRAS* mutation in adenocarcinomas of the lung [40, 47]; however, the relationship between genetic and epigenetic alterations in lung tumorigenesis remains unclear. To clarify the interaction of genetic and epigenetic alterations, we examined the mutations in *EGFR* exons 18–21 and *KRAS* exon 2 as well as the methylation status of $p16^{INK4a}$, *RASSF1A*, *APC*, *RARβ*, and *CDH13* genes using a methylation-specific PCR assay in 164 NSCLC samples and searched for possible correlations [42]. Our findings showed that: (1) the probability of having *EGFR* mutations was significantly lower in $p16^{INK4a}$ and *CDH13* methylated cases than in those without methylation, (2) the methylation index (MI; the number of methylated genes in a case) was significantly lower in *EGFR* mutant cases than in wild-type cases, (3) the probability of having *KRAS* mutations was significantly higher in $p16^{INK4a}$ methylated cases than in unmethylated cases, and (4) the MI was marginally higher in *KRAS* mutant cases than in wild-type cases. Of particular interest, *EGFR* mutations and $p16^{INK4a}$ methylation were mutually exclusive among the 164 NSCLC samples that were examined, except for two cases that exhibited both $p16^{INK4a}$ methylation and *EGFR* mutations (Fig. 3). These results suggest differences in the involvement of epigenetic alterations in *EGFR*- and *KRAS*-mediated tumorigenesis and suggest the specific interaction of genetic and epigenetic

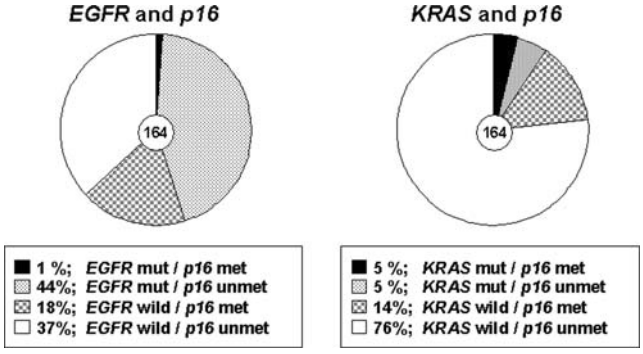

Fig. 3 Pie charts to show the relationship between the mutation of *EGFR* and *KRAS* and $p16^{INK4a}$ methylation. The *EGFR* mutation and $p16^{INK4a}$ methylation were mutually exclusive except for two cases (1%) that exhibited both *EGFR* mutation and $p16^{INK4a}$ methylation

changes in lung carcinogenesis (Fig. 4). Additional studies involving other genes are necessary to further our understanding of genetic and epigenetic interactions during lung carcinogenesis.

Conclusions and future perspectives

Considerable research on the role of *EGFR* in lung cancer has been conducted since the first reports by Lynch [24] and Paez [27] were published, and some points of consensus have been reached: (1) *EGFR* mutations are exclusively present in the tyrosine kinase domain encoded by exons 18–21, mainly in exons 19 and 21, (2) *EGFR* mutations cause the constitutive activation of *EGFR*, resulting in the up-regulation of oncogenic functions, (3) the frequency of *EGFR* mutations differs from one population to another, and (4) *EGFR* and *KRAS* mutations exhibit a mutually exclusive relationship. Our previous studies confirmed and contributed to these points of consensus. On the other hand, controversial results have also been reported, especially regarding the relationship between *EGFR* status and the tumor response to the EGFR-TKIs, gefitinib and erlotinib. In addition, the impact of *HER2* and *HER3* amplification on gefitinib-treated NSCLC has also been

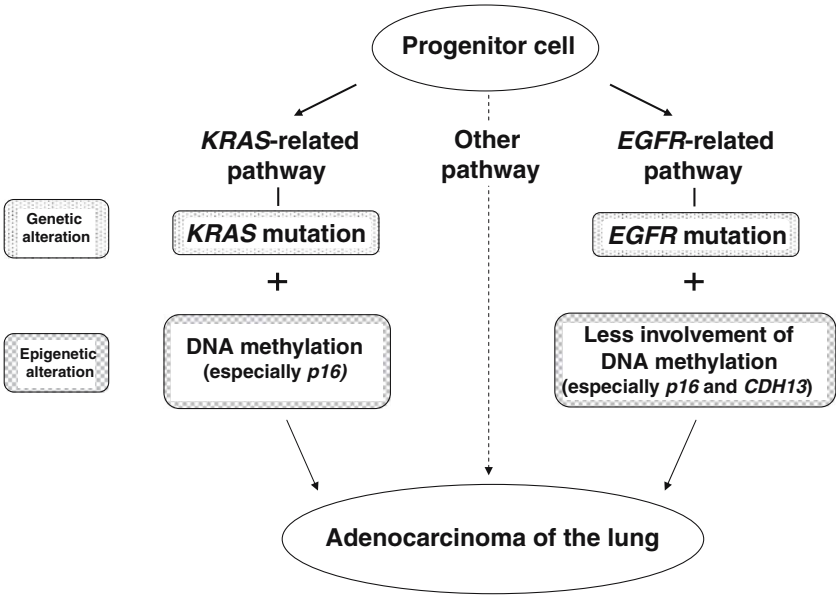

Fig. 4 The difference in the involvement of epigenetic alterations in the *EGFR*-related and *KRAS*-related pathways for lung adenocarcinoma

examined. A link between *HER2* amplification and a better clinical outcome in gefitinib-treated NSCLC has suggested that TKI sensitivity is not dependent solely on the presence of EGFR, but is also largely influenced by other family members [8, 9]. More recently, Fujimoto et al. reported that *EGFR* mutations are not sufficient to modulate the sensitivity of lung adenocarcinoma to gefitinib, but that the high expression of EGFR dimeric partners, especially ErbB3 and ErbB ligands (amphiregulin and epiregulin), are critical to tumor responsiveness to EGFR-TKIs [12, 33]. A comprehensive approach considering the *EGFR* status, including polymorphisms, as well as the status of ErbB family numbers will be essential for determining predictors of gefitinib response in NSCLC.

Although it is not discussed in this article and no consensus has been established, the mutagen for the *EGFR* gene is another critical issue requiring investigation. The identification of this mutagen will be important not only for understanding the mechanism of carcinogenesis, but also for establishing strategies to prevent *EGFR*-related lung cancer. To date, the only information we have is that tobacco smoking is not related to *EGFR* mutagenesis. Because the rate of adenocarcinoma in never smokers has been increasing worldwide [18, 30], the identification of a causative factor for *EGFR* mutation may reduce lung cancer mortality. Further studies, including an epidemiological approach, are required.

EGFR will be a key factor in the next stage of treatment and in furthering our understanding of lung carcinogenesis. Further knowledge of *EGFR* and its related members will be essential to the development of preventive and therapeutic strategies against lung cancer.

References

1. Asano H, Toyooka S, Tokumo M, Ichimura K, Aoe K, Ito S, Tsukuda K, Ouchida M, Aoe M, Katayama H, Hiraki A, Sugi K, Kiura K, Date H, Shimizu N (2006) Detection of EGFR gene mutation in lung cancer by mutant-enriched polymerase chain reaction assay. Clin Cancer Res 12:43–48
2. Baylin SB, Herman JG, Graff JR, Vertino PM, Issa JP (1998) Alterations in DNA methylation: a fundamental aspect of neoplasia. Adv Cancer Res 72:141–196
3. Bell DW, Lynch TJ, Haserlat SM, Harris PL, Okimoto RA, Brannigan BW, Sgroi DC, Muir B, Riemenschneider MJ, Bailey Iacona R, Krebs AD, Johnson DH, Giaccone G, Herbst RS, Manegold C, Fukuoka M, Kris MG, Baselga J, Ochs JS, Haber DA (2005) Epidermal growth factor receptor mutations and gene amplification in non-small-cell lung cancer: molecular analysis of the IDEAL/INTACT gefitinib trials. J Clin Oncol 23:8081–8092
4. Bos JL (1989) ras Oncogenes in human cancer: a review. Cancer Res 49:4682–4689
5. Buerger H, Gebhardt F, Schmidt H, Beckmann A, Hutmacher K, Simon R, Lelle R, Boecker W, Brandt B (2000) Length and loss of heterozygosity of an intron 1 polymorphic sequence of EGFR is related to cytogenetic alterations and epithelial growth factor receptor expression. Cancer Res 60:854–857
6. Buerger H, Packeisen J, Boecker A, Tidow N, Kersting C, Bielawski K, Isola J, Yatabe Y, Nakachi K, Boecker W, Brandt B (2004) Allelic length of a CA dinucleotide repeat in the EGFR gene correlates with the frequency of amplifications of this sequence—first results of an inter-ethnic breast cancer study. J Pathol 203:545–550
7. 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, Varella-Garcia M (2005) Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer. J Natl Cancer Inst 97:643–655
8. Cappuzzo F, Toschi L, Domenichini I, Bartolini S, Ceresoli GL, Rossi E, Ludovini V, Cancellieri A, Magrini E, Bemis L, Franklin WA, Crino L, Bunn PA Jr, Hirsch FR, Varella-Garcia M (2005) HER3 genomic gain and sensitivity to gefitinib

in advanced non-small-cell lung cancer patients. Br J Cancer 93:1334–1340
9. Cappuzzo F, Varella-Garcia M, Shigematsu H, Domenichini I, Bartolini S, Ceresoli GL, Rossi E, Ludovini V, Gregorc V, Toschi L, Franklin WA, Crino L, Gazdar AF, Bunn PA Jr, Hirsch FR (2005) Increased HER2 gene copy number is associated with response to gefitinib therapy in epidermal growth factor receptor-positive non-small-cell lung cancer patients. J Clin Oncol 23:5007–5018
10. Chou TY, Chiu CH, Li LH, Hsiao CY, Tzen CY, Chang KT, Chen YM, Perng RP, Tsai SF, Tsai CM (2005) Mutation in the tyrosine kinase domain of epidermal growth factor receptor is a predictive and prognostic factor for gefitinib treatment in patients with non-small cell lung cancer. Clin Cancer Res 11:3750–3757
11. Ciardiello F, De Vita F, Orditura M, Tortora G (2004) The role of EGFR inhibitors in nonsmall cell lung cancer. Curr Opin Oncol 16:130–135
12. Fujimoto N, Wislez M, Zhang J, Iwanaga K, Dackor J, Hanna AE, Kalyankrishna S, Cody DD, Price RE, Sato M, Shay JW, Minna JD, Peyton M, Tang X, Massarelli E, Herbst R, Threadgill DW, Wistuba II, Kurie JM (2005) High expression of ErbB family members and their ligands in lung adenocarcinomas that are sensitive to inhibition of epidermal growth factor receptor. Cancer Res 65:11478–11485
13. Fukuoka M, Yano S, Giaccone G, Tamura T, Nakagawa K, Douillard JY, Nishiwaki Y, Vansteenkiste J, Kudoh S, Rischin D, Eek R, Horai T, Noda K, Takata I, Smit E, Averbuch S, Macleod A, Feyereislova A, Dong RP, Baselga J (2003) Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer (The IDEAL 1 Trial). J Clin Oncol 21:2237–2246
14. Gazdar AF, Shigematsu H, Herz J, Minna JD (2004) Mutations and addiction to EGFR: the Achilles 'heal' of lung cancers? Trends Mol Med 10:481–486
15. Greulich H, Chen TH, Feng W, Janne PA, Alvarez JV, Zappaterra M, Bulmer SE, Frank DA, Hahn WC, Sellers WR, Meyerson M (2005) Oncogenic transformation by inhibitor-sensitive and -resistant EGFR mutants. PLoS Med 2:e313
16. Han SW, Kim TY, Hwang PG, Jeong S, Kim J, Choi IS, Oh DY, Kim JH, Kim DW, Chung DH, Im SA, Kim YT, Lee JS, Heo DS, Bang YJ, Kim NK (2005) Predictive and prognostic impact of epidermal growth factor receptor mutation in non-small-cell lung cancer patients treated with gefitinib. J Clin Oncol 23:2493–2501
17. Janne PA, Borras AM, Kuang Y, Rogers AM, Joshi VA, Liyanage H, Lindeman N, Lee JC, Halmos B, Maher EA, Distel RJ, Meyerson M, Johnson BE (2006) A rapid and sensitive enzymatic method for epidermal growth factor receptor mutation screening. Clin Cancer Res 12(3 Pt 1):751–758
18. Jemal A, Murray T, Ward E, Samuels A, Tiwari RC, Ghafoor A, Feuer EJ, Thun MJ (2005) Cancer statistics, 2005. CA Cancer J Clin 55:10–30
19. Kahn SM, Jiang W, Culbertson TA, Weinstein IB, Williams GM, Tomita N, Ronai Z (1991) Rapid and sensitive nonradioactive detection of mutant K-ras genes via 'enriched' PCR amplification. Oncogene 6:1079–1083
20. Kobayashi S, Boggon TJ, Dayaram T, Janne PA, Kocher O, Meyerson M, Johnson BE, Eck MJ, Tenen DG, Halmos B (2005) EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. N Engl J Med 352:786–792
21. Kosaka T, Yatabe Y, Endoh H, Kuwano H, Takahashi T, Mitsudomi T (2004) Mutations of the epidermal growth factor receptor gene in lung cancer: biological and clinical implications. Cancer Res 64:8919–8923
22. Liu W, Innocenti F, Chen P, Das S, Cook EH Jr, Ratain MJ (2003) Interethnic difference in the allelic distribution of human epidermal growth factor receptor intron 1 polymorphism. Clin Cancer Res 9:1009–1012
23. Liu W, Innocenti F, Wu MH, Desai AA, Dolan ME, Cook EH Jr, Ratain MJ (2005) A functional common polymorphism in a Sp1 recognition site of the epidermal growth factor receptor gene promoter. Cancer Res 65:46–53
24. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, Harris PL, Haserlat SM, Supko JG, Haluska FG, Louis DN, Christiani DC, Settleman J, Haber DA (2004) Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 350:2129–2139
25. Mitsudomi T, Kosaka T, Endoh H, Horio Y, Hida T, Mori S, Hatooka S, Shinoda M, Takahashi T, Yatabe Y (2005) 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 23:2513–2520
26. Nagai Y, Miyazawa H, Huqun, Tanaka T, Udagawa K, Kato M, Fukuyama S, Yokote A, Kobayashi K, Kanazawa M, Hagiwara K (2005) Genetic heterogeneity of the epidermal growth factor receptor in non-small cell lung cancer cell lines revealed by a rapid and sensitive detection system, the peptide nucleic acid-locked nucleic acid PCR clamp. Cancer Res 65:7276–7282
27. 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, Meyerson M (2004) EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 304:1497–1500
28. Pao W, Miller V, Zakowski M, Doherty J, Politi K, Sarkaria I, Singh B, Heelan R, Rusch V, Fulton L, Mardis E, Kupfer D, Wilson R, Kris M, Varmus H (2004) EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. Proc Natl Acad Sci USA 101:13306–13311
29. Pao W, Miller VA, Politi KA, Riely GJ, Somwar R, Zakowski MF, Kris MG, Varmus H (2005) Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. PLoS Med 2:e73
30. Parkin DM (2001) Global cancer statistics in the year 2000. Lancet Oncol 2:533–543
31. Riely GJ, Pao W, Pham D, Li AR, Rizvi N, Venkatraman ES, Zakowski MF, Kris MG, Ladanyi M, Miller VA (2006) Clinical course of patients with non-small cell lung cancer and epidermal growth factor receptor exon 19 and exon 21 mutations treated with gefitinib or erlotinib. Clin Cancer Res 12(3 Pt 1):839–844
32. Rusch V, Baselga J, Cordon-Cardo C, Orazem J, Zaman M, Hoda S, McIntosh J, Kurie J, Dmitrovsky E (1993) Differential expression of the epidermal growth factor receptor and its ligands in primary non-small cell lung cancers and adjacent benign lung. Cancer Res 53:2379–2385
33. Schlessinger J (2002) Ligand-induced, receptor-mediated dimerization and activation of EGF receptor. Cell 110:669–672
34. Shepherd FA, Tsao MS (2006) Unraveling the mystery of prognostic and predictive factors in epidermal growth factor receptor therapy. J Clin Oncol 24:1219–1220
35. 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, Gazdar AF (2005) Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. J Natl Cancer Inst 97:339–346

36. Shigematsu H, Toyooka S, Suzuki M (2006) The need to establish an optimal approach. PLoS Med 3 (in press)
37. Shih JY, Gow CH, Yang PC (2005) EGFR mutation conferring primary resistance to gefitinib in non-small-cell lung cancer. N Engl J Med 353:207–208
38. Takano T, Ohe Y, Sakamoto H, Tsuta K, Matsuno Y, Tateishi U, Yamamoto S, Nokihara H, Yamamoto N, Sekine I, Kunitoh H, Shibata T, Sakiyama T, Yoshida T, Tamura T (2005) Epidermal growth factor receptor gene mutations and increased copy numbers predict gefitinib sensitivity in patients with recurrent non-small-cell lung cancer. J Clin Oncol 23:6829–6837
39. Tokumo M, Toyooka S, Kiura K, Shigematsu H, Tomii K, Aoe M, Ichimura K, Tsuda T, Yano M, Tsukuda K, Tabata M, Ueoka H, Tanimoto M, Date H, Gazdar AF, Shimizu N (2005) The relationship between epidermal growth factor receptor mutations and clinicopathologic features in non-small cell lung cancers. Clin Cancer Res 11:1167–1173
40. Toyooka S, Maruyama R, Toyooka KO, McLerran D, Feng Z, Fukuyama Y, Virmani AK, Zochbauer-Muller S, Tsukuda K, Sugio K, Shimizu N, Shimizu K, Lee H, Chen CY, Fong KM, Gilcrease M, Roth JA, Minna JD, Gazdar AF (2003) Smoke exposure, histologic type and geography-related differences in the methylation profiles of non-small cell lung cancer. Int J Cancer 103:153–160
41. Toyooka S, Kiura K, Mitsudomi T (2005) EGFR mutation and response of lung cancer to gefitinib. N Engl J Med 352:2136
42. Toyooka S, Tokumo M, Shigematsu H, Matsuo K, Asano H, Tomii K, Ichihara S, Suzuki M, Aoe M, Date H, Gazdar AF, Shimizu N (2006) Mutational and epigenetic evidence for independent pathways for lung adenocarcinomas arising in smokers and never smokers. Cancer Res 66:1371–1375
43. Toyooka S, Yatabe Y, Tokumo M, Ichimura K, Asano H, Tomii K, Aoe M, Yanai H, Date H, Mitsudomi T, Shimizu N (2006) Mutations of epidermal growth factor receptor and K-ras genes in adenosquamous carcinoma of the lung. Int J Cancer 118:1588–1590
44. Toyota M, Ohe-Toyota M, Ahuja N, Issa JP (2000) Distinct genetic profiles in colorectal tumors with or without the CpG island methylator phenotype. Proc Natl Acad Sci USA 97:710–715
45. Tsao MS, Sakurada A, Cutz JC, Zhu CQ, Kamel-Reid S, Squire J, Lorimer I, Zhang T, Liu N, Daneshmand M, Marrano P, da Cunha Santos G, Lagarde A, Richardson F, Seymour L, Whitehead M, Ding K, Pater J, Shepherd FA (2005) Erlotinib in lung cancer—molecular and clinical predictors of outcome. N Engl J Med 353:133–144
46. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smits AM, Bos JL (1988) Genetic alterations during colorectal-tumor development. N Engl J Med 319:525–532
47. Zochbauer-Muller S, Fong KM, Virmani AK, Geradts J, Gazdar AF, Minna JD (2001) Aberrant promoter methylation of multiple genes in non-small cell lung cancers. Cancer Res 61:249–255