CLINICAL SCIENCE

Epidermal growth factor receptor and KRAS mutations in Brazilian lung cancer patients

Carlos E. Bacchi, Heloísa Ciol, Eduardo M. Queiroga, Lucimara C. Benine, Luciana H. Silva, Elida B. Ojopi

Consultoria em Patologia, Botucatu/SP, Brazil.

OBJECTIVE: Epidermal growth factor receptor is involved in the pathogenesis of non-small cell lung cancer and has recently emerged as an important target for molecular therapeutics. The *KRAS* oncogene also plays an important role in the development of lung cancer. The aim of this study was to evaluate the frequency of epidermal growth factor receptor and *KRAS* mutations in a population of Brazilian patients with non-small cell lung cancer.

METHODS: A total of 207 specimens from Brazilian patients with non-small cell lung cancer were analyzed for activating epidermal growth factor receptor and *KRAS* somatic mutations, and their associations with clinicopathological characteristics (including age, gender, ethnicity, smoking habits, and histological subtype) were examined.

RESULTS: We identified 63 cases (30.4%) with epidermal growth factor receptor mutations and 30 cases (14.6%) with *KRAS* mutations. The most frequent epidermal growth factor receptor mutation we detected was a deletion in exon 19 (60.3%, 38 patients), followed by an L858R amino acid substitution in exon 21 (27%, 17 patients). The most common types of *KRAS* mutations were found in codon 12. There were no significant differences in epidermal growth factor receptor or *KRAS* mutations by gender or primary versus metastatic lung cancer. There was a higher prevalence of *KRAS* mutations in the non-Asian patients. Epidermal growth factor receptor mutations were more prevalent in adenocarcinomas than in non-adenocarcinoma histological types. Being a non-smoker was significantly associated with the prevalence of epidermal growth factor receptor mutations, but the prevalence of *KRAS* mutations was significantly associated with smoking.

CONCLUSIONS: This study is the first to examine the prevalence of epidermal growth factor receptor and *KRAS* mutations in a Brazilian population sample with non-small cell lung cancer.

KEYWORDS: EGFR, KRAS, Lung cancer, Brazil, Mutation.

Bacchi CE, Ciol H, Queiroga EM, Benine LC, Silva LH, Ojopi EB. *Epidermal growth factor receptor and KRAS* mutations in Brazilian lung cancer patients. Clinics. 2012;67(5):419-424.

Received for publication on October 31, 2011; **First review completed on** December 13, 2011; **Accepted for publication on** January 4, 2012

E-mail: bacchi@conspat.com.br

Tel.: +55-14-31125900

INTRODUCTION

Lung cancer is the leading cause of cancer-related death in the world, accounting for more than one million deaths each year (1,2). Non-small cell lung carcinoma (NSCLC) is the primary subtype. It represents approximately 85% of all lung cancers and is classified into three histological subtypes: adenocarcinoma, squamous cell carcinoma, and large-cell carcinoma (3). Most patients in developed and underdeveloped countries with NSCLC are diagnosed with locally advanced metastatic disease (stages III-IV). Cytotoxic chemotherapy has made a considerable contribution to lung cancer treatment but has had little impact on patient survival. Despite regimens that include multiple treatment modalities, including surgery, radiation, and chemotherapy, the five-year overall survival rate is less than 14%, emphasizing the unsatisfactory clinical responses to the currently available treatments (4). Consequently, the prognosis remains poor for patients with locally advanced NSCLC and as low as three percent for patients with metastatic disease (5).

Epidermal growth factor receptor (EGFR) is critically involved in NSCLC pathogenesis and has recently emerged as an important target for molecular therapeutics. Two small-molecule EGFR tyrosine kinase inhibitors, gefitinib and erlotinib, have demonstrated good potential for treating lung cancer (6,7). Somatically active EGFR mutations are involved in the pathogenesis of a considerable subset of lung adenocarcinomas and sensitize these tumors to gefitinib and erlotinib treatment. In fact, when patients with *EGFR* mutations are treated with one of these agents, almost all have better progression-free survival than similarly treated patients with no *EGFR* mutations (7-9). This important association has led to the routine use of molecular tests to identify the lung cancer patients who

No potential conflict of interest was reported.

harbor *EGFR* mutations before initiating first-line therapy with tyrosine kinase inhibitors. *KRAS* mutations are found in 15 to 25% of lung cancer patients (10). KRAS is downstream in the EGFR tyrosine kinase pathway; therefore, tyrosine kinase-based treatment with gefitinib and erlotinib is ineffective when KRAS is constitutively activated (11,12).

Lung cancers with *EGFR* mutations are prevalent among young female patients, non-smokers with adenocarcinomas and Asians (9,13). The frequency of *EGFR* mutations varies from 27 to 60% in Asians, from 8 to 13% in Europeans, and from 12 to 16% in African and white Americans (14,15). *EGFR* mutations occur most frequently in exons 18 to 21. The most common mutations, small in-frame deletions in exon 19 (>50%) and L858R substitutions in exon 21 (40%), are reported to be the most closely associated with EGFR inhibitor therapy response (16,17). By contrast, *KRAS* mutations are strongly associated with smoking, and similar to *EGFR* mutations, their frequency varies by ethnicity. In Caucasians, 20 to 30% of lung adenocarcinomas harbor *KRAS* mutations, as opposed to 5 to 20% of lung adenocarcinomas in Asians (18). Approximately 97% of NSCLC *KRAS* mutations involve codons 12 and 13 of exon 2 (19). Interestingly, somatic *EGFR* and *KRAS* mutations are almost always mutually exclusive (20).

To the best of our knowledge, there are no data reporting the joint frequency of *EGFR* and *KRAS* mutations in any South American population. In the present study, we sought to evaluate the frequency of *EGFR* and *KRAS* mutations in a series of Brazilian patients with lung cancer and to assess the association between these mutations and clinicopathological characteristics.

MATERIALS AND METHODS

Patient selection

A total of 207 formalin-fixed, paraffin-embedded specimens from Brazilian patients with lung cancer were obtained from the files of the *Consultoria em Patologia*, a large reference pathology laboratory located in Botucatu, State of São Paulo, Brazil, from May 2007 to March 2011. All of the specimens were sent to the reference laboratory specifically for *EGFR* and *KRAS* mutation analysis. In all of the specimens, either *EGFR* and *KRAS* mutations were detected or the four *EGFR* exons (18,19,20,21) and exon 2 (codons 2 and 3) of *KRAS* were analyzed and included in this study. All five geographic regions of Brazil (56.5% from the Southeast, 15.5% from the Northeast, 14.5% from the South, 12.1% from the Midwest, and 1.4% from the North) were represented in the selected cases.

Hematoxylin and eosin (H&E) staining, and mucicarmine and/or PAS with diastase staining when necessary, was performed on 5-μm sections from all of the representative paraffin blocks and reviewed by two pathologists (CEB and EMQ). The tumors were then histologically classified according to 2004 WHO criteria (3). Clinical information, including age, gender, ethnicity, and smoking habits, was obtained from pathology requests directly to the patient's physician and/or from the pathologists involved in the original diagnoses.

This study was approved by the Department of Pathology Scientific Committee of the University of Sao Paulo School of Medicine and by the Ethical Committee for Research Projects of the Hospital das Clinicas da Universidade de São Paulo (''Comissão de Ética para Analise de Projetos de Pesquisa'', CAPPesq, protocol #118/11).

DNA extraction and mutational analysis of *EGFR* and *KRAS*

The H&E stained sections of all the tumor specimens underwent microdissection prior to DNA extraction from the formalin-fixed embedded tissues. DNA was extracted from the selected areas using the saline method. Exons 18, 19, 20, and 21 of the *EGFR* gene and exon 2 of the *KRAS* gene were amplified using PCR (Polymerase Chain Reaction). The primers used have been previously described by Shigematsu et al. (21). The PCR was performed in 25-μL reactions containing 100 ng of DNA, 100-mM Tris-HCl, 500-mM KCl (pH 8.3), 2-mM $MgCl_2$, 0.2-mM dNTPs, 0.15 μM of each primer, and 1 U of platinum Taq polymerase. The PCR reaction was performed on a PTC-200 MJ Research Thermal Cycler. The initial denaturation at 94℃ for five minutes was followed by forty cycles of denaturation at 94℃ for sixty seconds, annealing at 60℃ for thirty seconds, and extension at 72℃ for sixty seconds, with a final extension step of five minutes at 72℃. The amplified DNA was electrophoresed on a 7% polyacrylamide gel. The PCR products were sequenced directly in both directions using the BigDye® Terminator v3.1 (Applied Biosystems, Foster City, CA) sequencing ready reaction kit on the ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). Subsequent sequence analyses were performed using the Mutation Surveyor v3.9 (SoftGenetics, State College, PA, USA) and visual inspection. *KRAS* mutations (exon 2 and codons 12 and 13) were also detected using real-time PCR allelic discrimination. The PCR amplification was performed in 5-μL reactions with 5 ng of template DNA, 1x TaqMan Universal Master Mix (Applied Biosystems, Foster City, CA), 1x of each primer and a probe assay (Custom Taqman® SNP Genotyping assays), and H2O q.s.p. The thermal cycling was initiated with a denaturation step of ten minutes at 95℃, followed by 40 cycles of denaturation at 95℃ for 15 seconds and annealing at 60℃ for one minute on a 7500 Fast Real-Time System (Applied Biosystems, Foster City, CA).

Statistical analysis

The *EGFR* and *KRAS* mutation frequencies were compared using a proportions test with the normal approximation. The potential associations of *EGFR* and *KRAS* mutations with gender, ethnicity, smoking status, tumor histological subtype (adenocarcinomas versus non-adenocarcinoma), and metastatic versus primary tumors were analyzed using Chi-square statistics. A *p*-value less than 0.05 was considered to be statistically significant.

RESULTS

Clinicopathological characteristics of the patients

This series included 87 men and 120 women, and information on smoking habits was obtained for 162 patients (88 never-smokers and 74 current and ex-smokers). The study included 13 Asian and 194 non-Asian patients. One hundred sixty-nine cases were histologically classified as adenocarcinomas, and 38 cases were of non-adenocarcinoma types. One hundred sixty-one cases were primary tumors, and four were metastatic lung cancers.

Table 1 - A comparison of the frequency of *EGFR* and *KRAS* mutations by patient age (>45 and ≤45 years old).

Mutation			Age group (years old)		Total	Chi-squared (*p*-value)
			≤45	>45		
EGFR	No	n	13	123	136	1.183 (0.277)
		%	59.10%	70.70%	69.40%	
	Yes	n	9	51	60	
		%	40.90%	29.30%	30.60%	
KRAS	No	n	21	146	167	2.470 (0.116)
		%	95.50%	84.40%	85.60%	
	Yes	n	1	27	28	
		%	4.50%	15.60%	14.40%	

Table 3 - A comparison of *EGFR* and *KRAS* mutations in primary and metastatic lung cancers.

Mutation			Metastatic Tumor		Total	Chi-squared (*p*-value)
			No	Yes		
EGFR	**No**	n	112	32	144	0.000 (1.000)
		%	69.60%	69.60%	69.60%	
	Yes	n	49	14	63	
		%	30.40%	30.40%	30.40%	
KRAS	**No**	n	139	37	176	1.124 (0.289)
		%	86.90%	80.40%	85.40%	
	Yes	n	21	9	30	
		%	13.10%	19.60%	14.60%	

Types and Frequencies of *EGFR* and *KRAS* Mutations

The *EGFR* and *KRAS* mutational analyses were performed on 207 and 206 cases, respectively. Overall, 63 cases (30.4%) with *EGFR* mutations were identified, and 30 cases (14.6%) with *KRAS* mutations were identified. No cases of concomitant *EGFR* and *KRAS* mutations were identified. The most frequent *EGFR* mutation was an exon 19 deletion (60.3%, 38 patients), followed by an L858R amino acid substitution in exon 21 (27%, 17 patients). The following minor *EGFR* alterations were also detected: point mutations at codon 719 in exon 18 (7.9%, five patients); in-frame insertion mutations in exon 20 (4.8%, three patients); and one case with an L861Q substitution in exon 21. The most common *KRAS* mutations were observed in codon 12: G12C [GGC>TGT] in 15 cases (50%) and G12D [GGT>GAT] in 6 cases (20%).

Association of *EGFR* and *KRAS* mutations with clinicopathological factors

Information on patient age was obtained in 196 and 195 cases subjected to *EGFR* and *KRAS* mutational analysis, respectively, with a mean age of 62.8 (range 25-91). The age of the subjects was categorized into two groups (>45 and ≤45 years old) because lung cancer increases with age and because some studies have shown that *EGFR* mutations are more prevalent in younger lung cancer patients (9,13). There were no differences in the *EGFR* and *KRAS* mutational frequencies between the >45 and ≤45 year old groups (Table 1). There were also no statistically significant differences in gender or primary versus metastatic lung cancer by *EGFR* or *KRAS* mutation status (Tables 2 and 3). Although the *EGFR* mutational analyses found no difference between the Asian and non-Asian patients, *KRAS* mutations were much more prevalent in the non-Asian patients. In fact, all of the *KRAS* mutations (30 cases) identified were in non-Asian patients (Table 4). *EGFR* mutations were more prevalent in the adenocarcinomas than in the non-adenocarcinoma histological subtypes (33.7% versus 15.80%, respectively, Table 5). By contrast, the *KRAS* mutations did not differ significantly between these two groups. Smoking status information was obtained for 162 of the patients with *EGFR* mutations and 161 of the patients with *KRAS* mutations. Smoking status was significantly associated with *EGFR* mutations; 45.5% of the non-smokers had *EGFR* mutations, compared with 18.9% of the smokers. In addition, *KRAS* mutations were significantly associated smoking; the frequency of *KRAS* mutations was 2.7 times higher in the smokers (Table 6).

Table 2 - A comparison of *EGFR* and *KRAS* mutations in male and female Brazilian lung cancer patients.

Mutation			Gender		Total	Chi-squared (*p*-value)
			Male	Female		
EGFR	**No**	n	64	80	144	1.133 (0.287)
		%	73.60%	66.70%	69.60%	
	Yes	n	23	40	63	
		%	26.40%	33.30%	30.40%	
KRAS	**No**	n	72	104	176	0.868 (0.351)
		%	82.80%	87.40%	85.40%	
	Yes	n	15	15	30	
		%	17.20%	12.60%	14.60%	

DISCUSSION

The EGFR and its ligands are frequently over-expressed during NSCLC development and progression. The EGFR regulates important tumorigenic processes, including cell proliferation, apoptosis, angiogenesis, and invasion (17,22). In 2004, somatic mutations in the *EGFR* gene were identified in a subset of lung adenocarcinomas and were strongly associated with patient response to the EGFR tyrosine kinase inhibitors erlotinib and gefitinib (8,9,13). These studies found an 81% response rate in the patients harboring EGFR tyrosine kinase mutations (9), while less than 10% of the patients with wild-type EGFRs responded (23). By contrast, *KRAS* mutations have been shown to predict a poor response to EGFR tyrosine kinase inhibitor treatment (24).

Table 4 - The ethnic distribution (Asian versus non-Asian) of *EGFR* and *KRAS* mutations in Brazilian lung cancer patients.

Mutation			Asian		Total	Chi-squared (*p*-value)
			No	Yes		
EGFR	**No**	n	135	9	144	0.001 (0.978)
		%	69.60%	69.20%	69.60%	
	Yes	n	59	4	63	
		%	30.40%	30.80%	30.40%	
KRAS	**No**	n	163	13	176	4.239 (0.040)
		%	84.50%	100.00%	85.40%	
	Yes	n	30		30	
		%	15.50%		14.60%	

Table 5 - The association of histological subtype (adenocarcinoma versus non-adenocarcinoma) with *EGFR* and *KRAS* mutations in Brazilian lung cancer patients.

Mutation			Adenocarcinoma		Total	Chi-squared (*p*-value)
			No	Yes		
EGFR	**No**	n	32	112	144	4.715 (0.030)
		%	84.20%	66.30%	69.60%	
	Yes	n	6	57	63	
		%	15.80%	33.70%	30.40%	
KRAS	**No**	n	35	141	176	1.886 (0.170)
		%	92.10%	83.90%	85.40%	
	Yes	n	3	27	30	
		%	7.90%	16.10%	14.60%	

Given the high cost of EGFR tyrosine kinase inhibitor treatment and its potential side effects, there is an urgent need to determine the distributions of *EGFR* and *KRAS* mutations in different ethnic populations and perform a cost-benefit analysis for tyrosine kinase inhibitor treatment. Numerous clinical trials have confirmed the strong association between EGFR tyrosine kinase domain mutations and response to treatment with EGFR tyrosine kinase inhibitors in both Western and Asian populations (25). By contrast, the tyrosine kinase inhibitor response rates of wild-type EGFR lung tumors have been found to be low.

In the present study, we found that 30.4% (63+/207) and 14.6% (30+/206) of a Brazilian NSCLC patient population harbored *EGFR* and *KRAS* mutations, respectively. Interestingly, the *EGFR* mutation rate observed in this patient population was higher than that described for Europeans (8-13%) and Americans (10-16%) and close to the rate observed in Asians (30-50%). Additionally, although *EGFR* mutations seem to be associated with East Asian ethnicity, no geographic associations have been found, suggesting that *EGFR* mutations are related to genetic rather than environmental factors (13,21,26).

Brazil is the fifth largest country in the world. Its population of approximately 190 million people is distributed over five large geographic regions (macroregions), with marked heterogeneity in socioeconomic development, population density, and climate characteristics. The population of Brazil was formed by extensive admixtures between Amerindians, Europeans, Africans, and Asians. It is one of the most diverse populations in the world due to five centuries of ethnic blending involving populations from three continents. The patients in our study represent all five geographic regions of Brazil. Their history of ethnic blending may account for the 30.4% *EGFR* mutation rate we found. The 14.6% frequency of *KRAS* mutations in our study lies between the frequencies detected in Caucasians and Asians. In fact, a meta-analysis of 22 studies by Mao et al. (27) noted that *KRAS* mutations were detected in 231 of the 1470 analyzed patients (16%), which is similar to the rate observed in our study.

Table 6 - The association of smoking status (never smoked versus current or ex-smoker) with *EGFR* and *KRAS* mutations in Brazilian lung cancer patients.

Mutation			Smoking Status		Total	Chi-squared (*p*-value)
			No	Yes		
EGFR	**No**	n	48	60	108	12.737 (<0.001)
		%	54.50%	81.10%	66.70%	
	Yes	n	40	14	54	
		%	45.50%	18.90%	33.30%	
KRAS	**No**	n	81	60	141	5.313 (0.020)
		%	93.10%	81.10%	87.60%	
	Yes	n	6	14	20	
		%	6.90%	18.90%	12.40%	

In compiled data from multiple publications, 569 mutations were detected in 2880 lung cancer patients. The *EGFR* mutations in these studies were distributed as follows: 48.2% in exon 19, 42.7% in exon 21, 3.7% in exon 20, and 3.2% in exon 18 (28). In the present study, the most frequently detected *EGFR* mutation was a deletion in exon 19 (60.3%, 38 patients) followed by a L858R substitution in exon 21 (27%, 17 patients). The most common types of *KRAS* mutations we observed were in codon 12: G12C [GGC>TGT] in 15 (50%) cases and G12D [GGT>GAT] in 6 (20%) cases. Our data on *KRAS* mutations are consistent with that from other studies in the literature, in which a disproportionately high number of cysteine for glycine changes are described. Cysteine missense substitutions are the result of a G→T change in the first base of either codon 12 or 13. They have been attributed to the polycyclic aromatic hydrocarbons that are present in tobacco smoke.

According to several reports in the literature, *EGFR* lung cancers are more prevalent among young female patients who have never smoked, adenocarcinomas, and East Asians (9,13,21,32). By contrast, *KRAS* mutations occur mainly in smokers and adenocarcinoma patients (30,31). We divided our patients into two groups according to age, >45 and ≤45, and found no significant differences between these two groups for either *EGFR* or *KRAS* mutations. Although most studies have reported that *EGFR* mutations are more prevalent in young patients (9,13), others have been unable to find such an association. Sahoo et al. (32) categorized their 220 NSCLC Indian patients into three age groups: 20-40, 40-60, and >60. They found no significant differences in *EGFR* mutation prevalence for any of their age groups.

Although we found that *EGFR* mutations were more common in females than in males (33.3% versus 26.4%, respectively), we were unable to detect any significant differences in the *EGFR* or *KRAS* mutation prevalences by gender. This finding is somewhat inconsistent with most reported studies, especially for *EGFR* mutations (21,25).

Studies of *EGFR* lung tumors in both Western and Asian populations have consistently found them to be more common in patients who have never smoked than in former or current smokers (9,25,33). By contrast, the pooled results from an analysis of six studies (with a total of 718 NSCLC patients) by Mao et al. (27) found that *KRAS* mutations were more common in smokers than in non-smokers. Our group of NSCLC patients showed similar associations; *EGFR* mutations were significantly more common (45.5%) in non-smokers than in current and ex-smokers (18.9%), and smokers were 2.7 times more likely to have a *KRAS* mutation than were non-smokers. Although we did not analyze the association between years of smoking and *EGFR* mutations, it is well known that the probability of *EGFR* mutations is inversely associated with the number of pack years smoked (25). Additionally, a non-smoking or low-smoking history is the strongest predictor of *EGFR* mutations (34).

The *EGFR* mutations in our series did not differ significantly between the Asian and non-Asian patients. By contrast, *KRAS* mutations were more common in the non-Asian patients. In fact, all of the *KRAS* mutations (30 cases) in our study were detected in non-Asian patients. It is important to mention that the number of patients with Asian ancestry in our study may not have been sufficient to allow detecting a statistically significant difference in the *EGFR* mutation frequency by ethnicity.

EGFR mutations in lung cancer patients are strongly associated with adenocarcinoma histology (21,25,33) and have been found in no more than 3% of other NSCLC subtypes (21,35). *EGFR* mutations were more prevalent (33.7%) in our Brazilian NSCLC patients with adenocarcinomas than in those with other histological subtypes (15.80%). No significant differences in *KRAS* mutations between these two groups were detected. Interestingly, no more than 3% of the non-adenocarcinoma lung cancer patients in our study population had *EGFR* mutations, which reflects the relative scarcity of this mutation in non-adenocarcinoma histological subtypes.

Our failure to find any difference in either *EGFR* or *KRAS* mutations by primary versus metastatic lung cancer indicates that both primary lung tumors and their metastases are suitable specimens for selecting patients for EGFR tyrosine kinase inhibitor treatment.

In summary, this study is the first to examine the prevalence of *EGFR* and *KRAS* mutations in a South American population. It consisted of 207 Brazilian NSCLC patients from all of the geographic regions of Brazil.

ACKNOWLEDGMENTS

The authors thank Antonio Bruni for the statistical analysis.

AUTHOR CONTRIBUTIONS

Ciol H, Benine LC, and Silva LH were responsible for DNA extraction and sequencing of the PCR products and participated in the analysis and interpretation of the data. Queiroga EM and Bacchi CE were responsible for the immunohistochemistry and clinical data interpretation. Ojopi EB was responsible for the analysis and interpretation of the sequencing data. Bacchi CE and Ojopi EB conceived the study and participated in its design and coordination. All the authors contributed to the manuscript writing and approved its final version.

REFERENCES

1. Parkin DM. Global cancer statistics in the year 2000. Lancet Oncol. 2001;2(9):533-43, http://dx.doi.org/10.1016/S1470-2045(01)00486-7.
2. Jemal A, Murray T, Ward E, Samuels A, Tiwari RC, Ghafoor A, Feuer EJ, Thun MJ. Cancer statistics, 2005. CA Cancer J Clin. 2005;55(1):10-30, http://dx.doi.org/10.3322/canjclin.55.1.10.
3. Travis WD, Brambilla E, Müller-Hermelink HK, Harris CC. Pathology and genetics: tumours of the lung, pleura, thymus, and heart. World Health Organization Classification of Tumours. Lyon: IARC Press, 2004.
4. Spira A, Ettinger DS. Multidisciplinary management of lung cancer. N Engl J Med. 2004;350(4):379-92.
5. Ries LAG, Melbert D, Krapcho M, Stinchcomb DG, Howlader N, Horner MJ, et al (eds). SEER Cancer Statistics Review, 1975-2005, National Cancer Institute. Bethesda, MD, http://seer.cancer.gov/csr/1975_2005/, based on November 2007 SEER data submission, posted to the SEER web site, 2008.
6. Shepherd FA, Rodrigues Pereira J, Ciuleanu T, Tan EH, Hirsh V, Thongprasert S, et al. Erlotinib in previously treated non-small-cell lung cancer. N Engl J Med. 2005;353(2):123-32, http://dx.doi.org/10.1056/NEJMoa050753.
7. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med. 2009;361(10):947-57, http://dx.doi.org/10.1056/NEJMoa0810699.
8. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med. 2004;350(21):2129-39, http://dx.doi.org/10.1056/NEJMoa040938.
9. Pao W, Miller V, Zakowski M, Doherty J, Politi K, Sarkaria I, et al. 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 U S A. 2004;101(36):13306-11, http://dx.doi.org/10.1073/pnas.0405220101.
10. Capella G, Cronauer-Mitra S, Pienado MA, Perucho M. Frequency and spectrum of mutations at codons 12 and 13 of the c-K-ras gene in human tumors. Environ Health Perspect. 1991;93:125-31, http://dx.doi.org/10.1289/ehp.9193125.
11. Pao W, Wang TY, Riely GJ, Miller VA, Pan Q, Ladanyi M, et al. KRAS mutations and primary resistance of lung adenocarcinomas to gefitinib or erlotinib. PLoS Med. 2005;2(1):e17, http://dx.doi.org/10.1371/journal.pmed.0020017.
12. van Zandwijk N, Mathy A, Boerrigter L, Ruijter H, Tielen I, de Jong D, et al. EGFR and KRAS mutations as criteria for treatment with tyrosine kinase inhibitors: retro- and prospective observations in non-small-cell lung cancer. Ann Oncol. 2007;18(1):99-103.
13. Paez JG, Jänne PA, Lee JC, Tracy S, Greulich H, Gabriel S, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science. 2004;304(5676):1497-500, http://dx.doi.org/10.1126/science.1099314.
14. Ma BB, Hui EP, Mok TS. Population-based differences in treatment outcome following anticancer drug therapies. Lancet Oncol. 2010;11(1):75-84, http://dx.doi.org/10.1016/S1470-2045(09)70160-3.
15. Cote ML, Haddad R, Edwards DJ, Atikukke G, Gadgeel S, Soubani AO, et al. Frequency and type of epidermal growth factor receptor mutations in African Americans with non-small cell lung cancer. J Thorac Oncol. 2011;6(3):627-30, http://dx.doi.org/10.1097/JTO.0b013e31820a0ec0.
16. Jackman DM, Yeap BY, Sequist LV, Lindeman N, Holmes AJ, Joshi VA, et al. Exon 19 deletion mutations of epidermal growth factor receptor are associated with prolonged survival in non-small cell lung cancer patients treated with gefitinib or erlotinib. Clin Cancer Res. 2006;12(13):3908-14, http://dx.doi.org/10.1158/1078-0432.CCR-06-0462.
17. Herbst RS, Heymach JV, Lippman SM. Lung cancer. N Engl J Med. 2008;359(13):1367-80, http://dx.doi.org/10.1056/NEJMra0802714.
18. Tomizawa Y, Iijima H, Sunaga N, Sato K, Takise A, Otani Y, Tanaka S, Suga T, Saito R, Ishizuka T, Dobashi K, Minna JD, Nakajima T, Mori M. Clinicopathologic significance of the mutations of the epidermal growth factor receptor gene in patients with non-small cell lung cancer. Clin Cancer Res. 2005;11(19 Pt 1):6816-22, http://dx.doi.org/10.1158/1078-0432.CCR-05-0441.
19. Forbes S, Clements J, Dawson E, Bamford S, Webb T, Dogan A, et al. COSMIC 2005. Br J Cancer. 2006;94(2):318-22, http://dx.doi.org/10.1038/sj.bjc.6602928.
20. Ding L, Getz G, Wheeler DA, Mardis ER, McLellan MD, Cibulskis K, et al. Somatic mutations affect key pathways in lung adenocarcinoma. Nature. 2008;455(7216):1069-75, http://dx.doi.org/10.1038/nature07423.
21. Shigematsu H, Lin L, Takahashi T, Nomura M, Suzuki M, Wistuba II, et al. Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. J Natl Cancer Inst. 2005;97(5):339-46.
22. Sun S, Schiller JH, Gazdar AF. Lung cancer in never smokers—a different disease. Nat Rev Cancer. 2007;7(10):778-90, http://dx.doi.org/10.1038/nrc2190.
23. Riely GJ, Politi KA, Miller VA, Pao W. Update on epidermal growth factor receptor mutations in non-small cell lung cancer. Clin Cancer Res. 2006;12(24):7232-41, http://dx.doi.org/10.1158/1078-0432.CCR-06-0658.
24. Massarelli E, Varella-Garcia M, Tang X, Xavier AC, Ozburn NC, Liu DD, et al. KRAS mutation is an important predictor of resistance to therapy with epidermal growth factor receptor tyrosine kinase inhibitors in non-small-cell lung cancer. Clin Cancer Res. 2007;13(10):2890-6, http://dx.doi.org/10.1158/1078-0432.CCR-06-3043.
25. Tokumo M, Toyooka S, Kiura K, Shigematsu H, Tomii K, Aoe M, et al. The relationship between epidermal growth factor receptor mutations and clinicopathologic features in non-small cell lung cancers. Clin Cancer Res. 2005;11(3):1167-73.
26. Tsao AS, Tang XM, Sabloff B, Xiao L, Shigematsu H, Roth J, et al. Clinicopathologic characteristics of the EGFR gene mutation in non-small cell lung cancer. J Thorac Oncol. 2006;1(3):231-9.
27. Mao C, Qiu LX, Liao RY, Du FB, Ding H, Yang WC, et al. KRAS mutations and resistance to EGFR-TKIs treatment in patients with non-small cell lung cancer: a meta-analysis of 22 studies. Lung Cancer. 2010;69(3):272-8, http://dx.doi.org/10.1016/j.lungcan.2009.11.020.
28. Toyooka S, Matsuo K, Shigematsu H, Kosaka T, Tokumo M, Yatabe Y, et al. The impact of sex and smoking status on the mutational spectrum of epidermal growth factor receptor gene in non small cell lung cancer. Clin Cancer Res. 2007;13(19):5763-8, http://dx.doi.org/10.1158/1078-0432.CCR-07-0216.
29. Yatabe Y, Kosaka T, Takahashi T, Mitsudomi T. EGFR mutation is specific for terminal respiratory unit type adenocarcinoma. Am J Surg Pathol. 2005;29(5):633-9.

30. Slebos RJ, Hruban RH, Dalesio O, Mooi WJ, Offerhaus GJ, Rodenhuis S. Relationship between K-ras oncogene activation and smoking in adenocarcinoma of the human lung. J Natl Cancer Inst. 1991;83(14):1024-7.
31. Ahrendt SA, Decker PA, Alawi EA, Zhu Yr YR, Sanchez-Cespedes M, Yang SC, et al. Cigarette smoking is strongly associated with mutation of the K-ras gene in patients with primary adenocarcinoma of the lung. Cancer. 2001;92(6):1525-30, http://dx.doi.org/10.1002/1097-0142(20010915)92:6<1525::AID-CNCR1478>3.0.CO;2-H.
32. Sahoo R, V VH, Babu VC, V Patil Okaly G, Rao S, Nargund A, et al. Screening for EGFR mutations in lung cancer, a report from India. Lung Cancer. 2011 Feb 9. [Epub ahead of print].
33. Tam IY, Chung LP, Suen WS, Wang E, Wong MC, Ho KK, et al. Distinct epidermal 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(5):1647-53, http://dx.doi.org/10.1158/1078-0432.CCR-05-1981.
34. Dacic S. EGFR assays in lung cancer. Adv Anat Pathol. 2008;15(4):241-7, http://dx.doi.org/10.1097/PAP.0b013e31817bf5a9.
35. Yang SH, Mechanic LE, Yang P, Landi MT, Bowman ED, Wampfler J, et al. Mutations in the tyrosine kinase domain of the epidermal growth factor receptor in non-small cell lung cancer. Clin Cancer Res. 2005;11(6):2106-10, http://dx.doi.org/10.1158/1078-0432.CCR-04-1853.