Med Oncol (2014) 31:87
DOI 10.1007/s12032-014-0087-4

ORIGINAL PAPER

EGFR and *KRAS* mutations in Turkish non-small cell lung cancer patients: a pilot study

Sema Bircan · Huseyin Baloglu · Zafer Kucukodaci · Ahmet Bircan

Received: 6 May 2014 / Accepted: 16 June 2014 / Published online: 29 June 2014

Abstract *EGFR* and *KRAS* mutation profile in non-small cell lung cancers (NSCLCs) shows wide variations due to geographic and ethnic background. We aimed to determine the frequency and types of *EGFR* and *KRAS* mutations in a sample group of Turkish NSCLC cases. The study included 14 adenocarcinomas (ACs), 11 squamous cell carcinoma (SCC) patients selected from archival material including small biopsy or surgical specimens. Their formalin fixed paraffin-embedded tumor tissues were used for genomic DNA extraction for *EGFR* exon 19 and 21, and *KRAS* exon 2 mutations. Eleven NSCLCs (44 %) had *EGFR* mutations. Exon 19 and 21 mutations were found in 8 (32 %) and 5 (20 %) cases. Two cases showed double *EGFR* mutations. In ACs, 5 (35.7 %) patients had *EGFR* gene mutation, 3 in exon 19 and 3 in exon 21. In SCCs, 6 (54.5 %) cases had *EGFR* mutation, 5 in exon 19 and 2 in exon 21. All exon 19 mutations were deletion-type mutations. For exon 21, 3 cases had L858R point mutation (CTG>CGG) and two cases showed deletion-type mutations. Six (24 %) NSCLCs showed *KRAS* mutations (three ACC, three SCC), 5 codon 12 mutations (G>T, T>C, G>A) and one codon 13 mutation (G>T). Three NSCLC cases showed both *EGFR* and *KRAS* mutations together. The profile of *KRAS* mutation in our AC cases was quite similar to those seen in the Western countries; however, frequency and clustering of *EGFR* mutations were similar to those seen in the Eastern countries.

Keywords Non-small cell lung cancer · *EGFR* · *KRAS* · Mutation · DNA extraction

S. Bircan (✉)
Department of Pathology, School of Medicine, Suleyman Demirel University, Isparta, Turkey
e-mail: bircans2000@yahoo.com

H. Baloglu
Department of Pathology, Anadolu Medical Center, Gebze, Turkey

Z. Kucukodaci
Department of Pathology, Gulhane Medical Academy Haydarpasa Teaching Hospital, Istanbul, Turkey

A. Bircan
Department of Chest Disease, School of Medicine, Suleyman Demirel University, Isparta, Turkey

Introduction

Lung cancer is the leading cause of cancer-related death in many countries, and non-small cell lung cancers (NSCLCs) account for approximately 80–85 % of lung cancers. Surgery is the most effective treatment; however, only small portion of total NSCLC cases is operable. Platinum-based chemotherapy is the standard therapy for advanced NSCLCs, but its results are still unsatisfactory [1, 2]. As a new therapeutic strategy, activating mutation of epidermal growth factor receptor (*EGFR*) is one of the most intriguing discoveries in the field of lung cancer research because *EGFR* is frequently aberrantly activated in NSCLCs [1, 3]. *EGFR*, a receptor tyrosine kinase (TK), plays essential roles in cell differentiation, development, proliferation and maintenance in both normal physiological and cancerous conditions [2–4]. The presence of activating *EGFR* mutations within TK domain was discovered in 2004 in a subgroup of NSCLC, and those patients were highly sensitive to EGFR-tyrosine kinase inhibitors (TKIs) [1, 5–8]. EGFR-TKI treatment is currently approved for NSCLC patients with sensitizing *EGFR* mutations in the first-line setting and also in the second-/third-line setting after

chemotherapy failure [9–11]. It is also intriguing that *EGFR* mutations are almost exclusively seen in lung cancer and not in other tumors [4].

Many reports have described that *EGFR* mutations are predominantly occurred in patients with adenocarcinoma, non-smokers, females and East Asian origin. It can be detected in about 20–50 % of Asian patients, while in about 10–15 % of Western patients with lung adenocarcinoma [6, 9, 12–17]. *EGFR* mutations are mainly present in the first four exons (18–21) encoding TK domain, and about 90 % of these mutations are small deletions in exon 19 and point mutation at codon 858 (L858R) in exon 21 [1, 3, 4, 6, 9, 12, 13, 18–20]. These mutations lead to the constitutive autophosphorylation and activation of *EGFR* and activate downstream signal transduction pathways [1, 2]. Increasing catalytic activity in intracellular TK domain of *EGFR* results in cell proliferation and differentiation, invasion, metastasis, angiogenesis and inhibition of apoptosis, which all are associated with cancer [2–4]. These effects are mediated by the activation of several downstream signal transduction cascades including Ras-Raf-mitogen-activated protein kinase (MAPK), Akt/PI3K, STAT pathways [2–4, 20].

KRAS gene is also important issue regarding tumorigenesis of lung [1]. *KRAS* gene encodes GTPase activity proteins that regulate cell growth, differentiation, apoptosis and involved in lung cancer development. It is known that *KRAS* proteins acquire transforming potential as a result of a point mutation at codon 12, 13 or 61, and these mutations lead to constitutive activation of RAS signaling due to impaired GTPase activity [18, 21]. GTP-bound active RAS binds multiple effectors proteins, more than 20, and stimulates downstream signaling cascades including MAPK, STAT pathways [6, 21]. *KRAS* is a downstream mediator of *EGFR*-induced cell signaling, and its mutations confer constitutive activation of the signaling pathways without *EGFR* activation [3]. *KRAS* mutations are detected in 15–30 % of NSCLC and particularly in adenocarcinomas in Western patients rather than East Asians and more frequent in smokers in contrast to *EGFR* mutations [6, 16, 18, 22, 23]. Approximately 97 % of *KRAS* mutations in NSCLC involve a point mutation in codons 12 or 13 in exon 2 [21].

Although genetic alterations of both *EGFR* and *KRAS* genes are important in the pathogenesis of NSCLC, and interestingly, the mutations of *EGFR* and *KRAS* are reported to be mutually exclusive and to have an inverse relationship in NSCLC [1, 24]. *EGFR* mutations are associated with sensitivity to TKIs and longer survival than those with *KRAS* mutations, while mutations in *KRAS* are associated with primary resistance and poor survival. Thus, these two mutations define distinct populations of patients with NSCLC with different responses to targeted therapy [21].

Mutation profile of *EGFR* and *KRAS* in NSCLC shows wide variations because of many factors including geographic and ethnic background, and there is no data about *EGFR* and *KRAS* gene status of Turkish NSCLC patients in the literature. Therefore, in the current study, we aimed to identify the genomic mutational status of *KRAS* gene and *EGFR* exon 19 and 21 gene mutations in a group of Turkish NSCLC patients and to compare our data with the available literature.

Materials and methods

The patients and methods

The study included primary NSCLC patients who proven the diagnosis as pathological. For the study, department of pathology archival files were reviewed retrospectively, and the hematoxylin–eosin-stained slides of the patients diagnosed as NSCLC were re-evaluated by light microscopy. The cases that had clearly lung adenocarcinoma (AC) and squamous cell carcinoma (SCC) morphologically were selected for the study. Finally, 25 NSCLC patients including 14 AC and 11 SCC were obtained for this study. All materials were formalin fixed paraffin-embedded (FFPE) tissues and included small biopsies such as transbronchial biopsy or trucut biopsy and also resection materials. The percentage of cancer cells was also determined in their hematoxylin–eosin-stained slides by light microscope. Finally, the tumoral content was ranged from 20 to 80 % in all cases. All these tumor samples had been obtained before chemotherapy and/or radiotherapy. Medical information of the patients including clinical stage and smoking status was obtained from hospital archival files.

DNA extraction and mutational analysis

Genomic DNA was obtained from primary tumoral tissues. The mutational analysis of exon 19 and 21 of *EGFR* gene, and exon 2 of *KRAS* gene was performed using polymerase chain reaction (PCR)-based direct sequencing. Genomic DNA was extracted from FFPE tissue sections which were five sections with 10 μm thick according to the manufacturer's instructions (QIAmp DNA FFPE tissue kit Qiagen, Valencia, CA). Extracted DNA was amplified by PCR for exon 19 and 21 of *EGFR* gene and exon 2 for *KRAS* gene. The PCRs were performed in a total volume of 25-μl reaction mixture (PCR master mix plus kit Qiagen, Valencia, CA) containing 30–50 ng genomic DNA and 0.2 μmol/L of each primer. The PCR cycle conditions were carried out in a Thermal Cycler consisting of initial denaturation step at 94 °C for 5 min, followed by 35 cycles of 30 s at 94 °C (denaturation), 1 min at 56 °C (annealing)

and 20 s at 72 °C (extension), and then a final elongation at 72 °C for 10 min. For *EGFR* exon 19 "5′-CGTCTTCCT TCTCTCTCTGTCA-3′" forward, "5′-GACCCCCACACA GCAAAG-3′" reverse; for exon 21 "5′-CCTCACAGCAG GGTCTTCTCTGT-3′" forward, "5′-TCAGGAAAATGC TGGCTGACCTA-3′" reverse; for *KRAS* exon 2 "5′-GTG TGACATGTTCTAATATAGTCA-3′" forward and "5′-CT GTATCAAAGAATGGTCCTGCAC-3′" reverse primers were used. The final precise amplicons were visualized on an agarose gel plague after electrophoresis. Each amplicon was purified and submitted to bidirectional conventional Sanger sequencing (Dye Terminator Cycle Sequencing, ABI PRISM 3700, Applied Biosytems, Foster City, CA, USA).

Results

The characteristics of the 25 NSCLC patients are shown in Tables 1 and 2. The mean age was 65.3 years in all patients (range 28–85 years). There were 21 (84 %) men and 4 (16 %) female in all NSCLC cases. Four (28.6 %) patients were females, and 10 (71.4 %) patients were males in AC patients, while all SCC cases were male gender. Six (26.1 %) NSCLC patients including 5 (41.7 %) AC and 1 (9.1 %) SCC were non-smokers, and remaining 17 patients (seven AC, ten SCC) were ex- or current smokers. The clinical stages of disease were distributed between stage II (five patients), IIIA (two patients), IIIB (six patients) and IV (ten patients). The diagnoses of 17 patients were obtained from small lung biopsy material such as trucut and transbronchial biopsy and remaining 8 patients underwent surgical procedures.

As showed in Tables 1 and 2, *EGFR* mutations were found in 11 (44 %) of NSCLC cases. Eight (32 %) cases showed exon 19 mutation, and 5 (20 %) cases had exon 21 mutations of *EGFR* gene. Two patients showed double *EGFR* exon 19 and 21 mutations. In AC patients, 5 (35.7 %) of 14 patients had a mutation in the *EGFR* gene, 3 in exon 19 and 3 in exon 21. One patient showed both exon 19 and 21 deletion mutations (Table 2). In SCC group, *EGFR* mutations were found in 6 (54.5 %) cases, of which 5 (45.5 %) in exon 19 and 2 (18.2 %) in exon 21. One SCC patient (Table 2) had both exon 19 and 21 deletion mutations together. Exon 19 mutations were frame or frame shift deletion-type mutations concerning codons 730–760 in both AC and SCC patients. Exon 21 mutations of three patients (2 AC, 1 SCC) were L858R (CTG>CGG), and remaining two patients (one AC, one SCC) showed deletion mutation at codons 839–840 and 847–849 in exon 21. Considering smoking status, in SCCs, only one patient with *EGFR* mutation had non-smoking history, while all others were ex- or current smokers. In ACs, three of five patients with *EGFR* gene mutation had non-smoking history, and other two patients were ex-smoker (Table 2). According to the *EGFR* mutation type, all of three AC patients with exon 21 mutation were never smoker; one of 3 exon 19 mutations had non-smoking history. Considering clinical stage, three ACs with *EGFR* exon 21 mutations were stage IV, while exon 19 mutations were distributed in all stages (Table 3).

Table 1 *EGFR* and *KRAS* mutations in patients with lung cancer

	NSCLC $n = 25$ (%)	AC $n = 14$ (%)	SCC $n = 11$ (%)
Age	28–85	28–85	43–79
Mean	65.3	65.8	64.6
Gender			
Female	4 (16)	4 (28.6)	–
Male	21 (84)	10 (71.4)	11 (100)
Smoking			
Never	6 (26.1)	5 (41.7)	1 (9.1)
Smoke	17 (73.9)	7 (58.3)	10 (90.9)
Stage			
II	5 (21.7)	2 (15.4)	3 (30)
III	8 (34.8)	2 (15.4)	6 (60)
IV	10 (43.5)	9 (69.2)	1 (10)
EGFR			
Mutant	11 (44)	5 (35.7)	6 (54.5)
Wild type	14 (56)	9 (64.3)	5 (45.5)
EGFR 19			
Mutant	8 (32)	3 (21.4)	5 (45.5)
Wild type	17 (68)	11 (78.6)	6 (54.5)
EGFR 21			
Mutant	5 (20)	3 (21.4)	2 (18.2)
Wild type	20 (80)	11 (78.6)	9 (81.8)
KRAS			
Mutant	6 (24)	3 (21.4)	3 (27.3)
Wild type	19 (76)	11 (78.6)	8 (72.7)

Considering *KRAS* gene, 6 (24 %) of NSCLC cases showed *KRAS* mutation (Table 1). These mutations were found in three AC (21.4 %) and three SCC (27.3 %) patients. Five patients (three AC, two SCC) showed codon 12 mutations (G>T, T>C, G>A), and only one SCC case had codon 13 mutation (G>T) (Fig. 1). All three SCCs and one AC with *KRAS* mutations (G>T, T>C) had ex- or current smoking history, and two AC cases with G>T and G>A mutation were non-smoker (Table 2). Interestingly, three NSCLC cases (two AC, one SCC) showed both *EGFR* and *KRAS* mutations. Two of them showed exon 21 point mutation (L858R) and *KRAS* codon 12 (GGT→GTT) mutations. The other case had multiple deletion mutation in exon 19 and codon 12 (GGT→GGC) mutation (Table 2).

Table 2 *EGFR* and *KRAS* gene mutations and clinicopathologic features in all 25 NSCLC patients

No	Age	Sex	Tissue	Diagnosis	Smoke	Stage	*EGFR* Exon 19	*EGFR* Exon 21	*KRAS*
1	79	F	Trucut	AC	Never	IV	Wt	L858R: CTG>CGG	Wt
2	61	M	Wedge	AC	Never	IV	del 753–760	del 847–849	Wt
3	61	M	Lobectomy	AC	Cur-Sm	IIIA	Wt	Wt	Wt
4	81	M	Biopsy	AC	Never	IV	Wt	L858R: CTG>CGG	Codon 12: GGT→GTT
5	66	M	Biopsy	AC	Ex-Sm	IV	del 729, del 731, del 741, del 751–753	Wt	Codon 12: GGT→GGC
6	61	M	Biopsy	AC	Cur-Sm	IV	Wt	Wt	Wt
7	67	M	Biopsy	AC	Cur-Sm	IIIB	Wt	Wt	Wt
8	85	F	Trucut	AC	Never	IV	Wt	Wt	Codon 12: GGT→AGT
9	77	M	Trucut	AC	Ex-Sm	IIA	del 743–747	Wt	Wt
10	70	F	Trucut	AC	UK	UK	Wt	Wt	Wt
11	52	M	Trucut	AC	Cur-Sm	IV	Wt	Wt	Wt
12	28	M	Lobectomy	AC	UK	IIB	Wt	Wt	Wt
13	76	F	Biopsy	AC	Never	IV	Wt	Wt	Wt
14	57	M	Lobectomy	AC	Cur-Sm	IV	Wt	Wt	Wt
15	43	M	Wedge	SCC	Never	IIB	del 757–760	Wt	Wt
16	50	M	Pnomonectomy	SCC	Cur-Sm	IIIA	Wt	Wt	Codon 13: GGC→TGC
17	75	M	Lobectomy	SCC	Ex-Sm	IIA	Wt	Wt	Codon 12: GGT→TGT
18	60	M	Pnomonectomy	SCC	Cur-Sm	IIB	del 734–736	Wt	Wt
19	67	M	Biopsy	SCC	Cur-Sm	IIIB	Wt	Wt	Wt
20	47	M	Biopsy	SCC	Cur-Sm	IIIB	Wt	Wt	Wt
21	72	M	Biopsy	SCC	Ex-Sm	IIIB	del 739–744	Wt	Wt
22	72	M	Biopsy	SCC	Cur-Sm	IIIB	del 731–736	Wt	Wt
23	77	M	Biopsy	SCC	Cur-Sm	IV	del 750–752	del 839–840	Wt
24	79	M	Biopsy	SCC	Ex-Sm	UK	Wt	L858R: CTG>CGG	Codon 12: GGT→GTT
25	69	M	Biopsy	SCC	Cur-Sm	IIIB	Wt	Wt	Wt

F female, *M* male, *AC* adenocarcinoma, *SCC* squamous cell carcinoma, *Never* non-smoker, *Ex-Sm* ex-smoker, *Cur-Sm* current smoker, *UK* unknown, *Wt* wild type

Discussion

One of the major molecular alterations in the carcinogenesis of NSCLC is the activation mutation of *EGFR* [2]. Many studies have reported that *EGFR* mutations are most frequently associated with sensitivity to EGFR TKIs [1–4, 6–8, 19, 25]. About 70–80 % of patients with *EGFR* mutations respond to *EGFR* TKIs [1, 6, 19]. Several reports indicate that patients with *EGFR* mutations have a significantly longer survival than patients with wild-type *EGFR* when treated with TKIs. However, some investigators claim that the *EGFR* mutations are only prognostic rather than a predictive factor [1, 6, 19].

The incidence of *EGFR* mutations in NSCLC is dependent upon tumor type, gender, smoking history and ethnic background [2]. It is frequently seen especially in adenocarcinoma, female, non-smokers and East Asian ethnicity patients. Its frequency is highly dependent on ethnicity, and it can be detected in about 20 to 50–65 % of East Asians patients in contrast to 10–15 % in Western patients [6, 9, 12–18, 23–25]. The studies have reported that *EGFR* mutations are much more frequently detected in ACs in comparison with other NSCLC type [2, 6, 12, 14, 15, 18, 20, 23, 24]. Tanaka et al. [12] found that *EGFR* mutation rate was 12 % in other NSCLC patients, while 43 % in adenocarcinomas. Park et al. [26] found *EGFR*

Table 3 The association of *EGFR* exon 19 and exon 21 mutations with smoking and clinical stage in AC and SCC

	$n = 25$	Exon 19 positive/negative		Exon 21 positive/negative	
		AC $n = 3/11$	SCC $n = 5/6$	AC $n = 3/11$	SCC $n = 2/9$
Smoking					
Never	6	1/4	1/–	3/2	–/1
Smoker	17	2/5	4/6	–/7	2/8
Unknown	2	/2		/2	
Stage					
II	5	1/1	2/1	–/2	–/3
III	8	–/2	2/4	–/2	–/6
IV	10	2/7	1/–	3/6	1/–
Unknown	2	/1	/1	/1	1/

mutations in 15 % of SCC cases. They reported that SCCs were not ideal candidate for TKI treatment; however, significant incidence of *EGFR* mutations was observed. In our study, the *EGFR* mutation rate was 44 % in all NSCLC, 35.7 % in ACC and 54.5 % in SCC patients. Its rates in NSCLCs and ACs were quite similar to those reported from East Asia. On the other hand, it was very high in SCCs in contrast to other studies. Although they had clearly SCC morphology by light microscopy, most of our SCC patients with *EGFR* mutations were diagnosed by small biopsies. Therefore, we can suggest that SCC patients should have to routinely test for *EGFR* mutations especially in small biopsies since the tumor may contain another histological component. Ladanyi and Pao [20] reported *EGFR* mutations in adenosquamous carcinomas, but it was extremely rare in other pure NSCLCs. Li et al. [15] found highest mutation ratio in ACs (51.5 %) and lowest in SCCs (8.3 %); however, they showed in 50 % of adenosquamous carcinoma. In addition, many studies have reported that ACs with bronchioloalveolar carcinoma (BAC) component, non-mucinous component, well-differentiated grades, hobnail cell type and micropapillary morphology and also terminal respiratory unit type adenocarcinoma that are associated with high incidence of *EGFR* mutations and might be good candidates for EGFR TKIs [2–4, 13, 16, 20, 27]. However, a subset of patients treated with EGFR-TKI, about half of NSCLC, develop resistance to TKI due to second-site mutation within the *EGFR* kinase domain such as T790M [1–3, 19]. Other acquired resistance to EGFR-TKI treatment is associated with *MET* amplification, histological transformation from NSCLC to SCLC and epithelial to mesenchymal transition [28].

Approximately 90 % of *EGFR* gene mutations are deletion type around codons 746–750 in exon 19 and a point mutation of codon 858 (L858R, G to T transversion) in exon 21. Other less common mutations include point mutations in exon 18 (G719X) (3 %) and in-frame insertion in exon 20 (3 %) [1, 3, 4, 6, 9, 12, 13, 18–20]. There are over 20 variant types of deletion in exon 19 [1, 4, 6, 19]. They cause conformational change in the ATP-binding domain which results in constant activation of *EGFR* without ligand binding. As a result, several downstream pathways promoted by constitutive activation of *EGFR* activate signal transduction, cell differentiation, proliferation and survival [2–4, 19]. In our study, the rate of *EGFR* exon 19 mutations was higher than exon 21 in NSCLC (32 vs. 20 %) and also in SCC patients (45.5 vs. 18.2 %), although they were the same in ACs (21.4 %). The mutation characteristics of the cases were similar to other reported data. Exon 19 mutations were mostly deletion type around codons 730–760, and exon 21 mutations included mostly L858R transversion mutation of T to G. In addition, as reported in some studies [12, 24], we found exon 19 and 21 mutations simultaneously in three cases. Furthermore, exon 21 mutations were more prevalent in ACs patients with non-smoking history and also advanced stage. The studies have reported that exon 19 deletions confer higher malignant transforming ability than the exon 21 point mutation, and also the response rate to TKIs is highest in exon 19 deletions [1]. However, Li et al. [15] reported that the stages III and IV had significantly higher exon 21 mutation frequency, while exon 19 mutations were more in the tumors with lower stage. It was also reported that exon 21 mutations were higher in female gender [12, 15]. Furthermore, studies have reported that exon 18 and 19 mutations are associated with better survival [9, 17, 20]. Although our study included limited number of cases, all these findings may consider that exon 19 and 21 mutations have different clinical and prognostic features.

On the other hand, *KRAS* gene mutation is an important issue regarding tumorigenesis of lung [1]. Activating mutation of *KRAS* gene is one of the earliest discoveries of genetic alterations in lung cancer [19], and about 15–30 % of NSCLCs harbor activating *KRAS* mutation [2, 6, 18, 24]. They are more common in AC than other histological subtypes [14, 15, 18, 21, 22, 24]. *KRAS* mutations are associated with poor prognosis in lung ACs [6, 18, 22], but data are contradictory [29]. In addition, *KRAS* mutation is an important predictor of primary resistance to EGFR-TKI therapy [9, 20, 25, 30], and the response rate of these patients is virtually zero [6].

KRAS mutation occurs predominantly in Caucasian patients (15–30 %), while it is approximately up to 10–15 % in East Asians [6, 14–17, 22, 23, 25]. Bae et al. [14] found *KRAS* mutation in 7.3 % of AC and 3.3 % of SCC cases. Wang et al. [24] did not find any *KRAS* gene mutation in 24 Chinese NSCLC patients. *KRAS* mutations

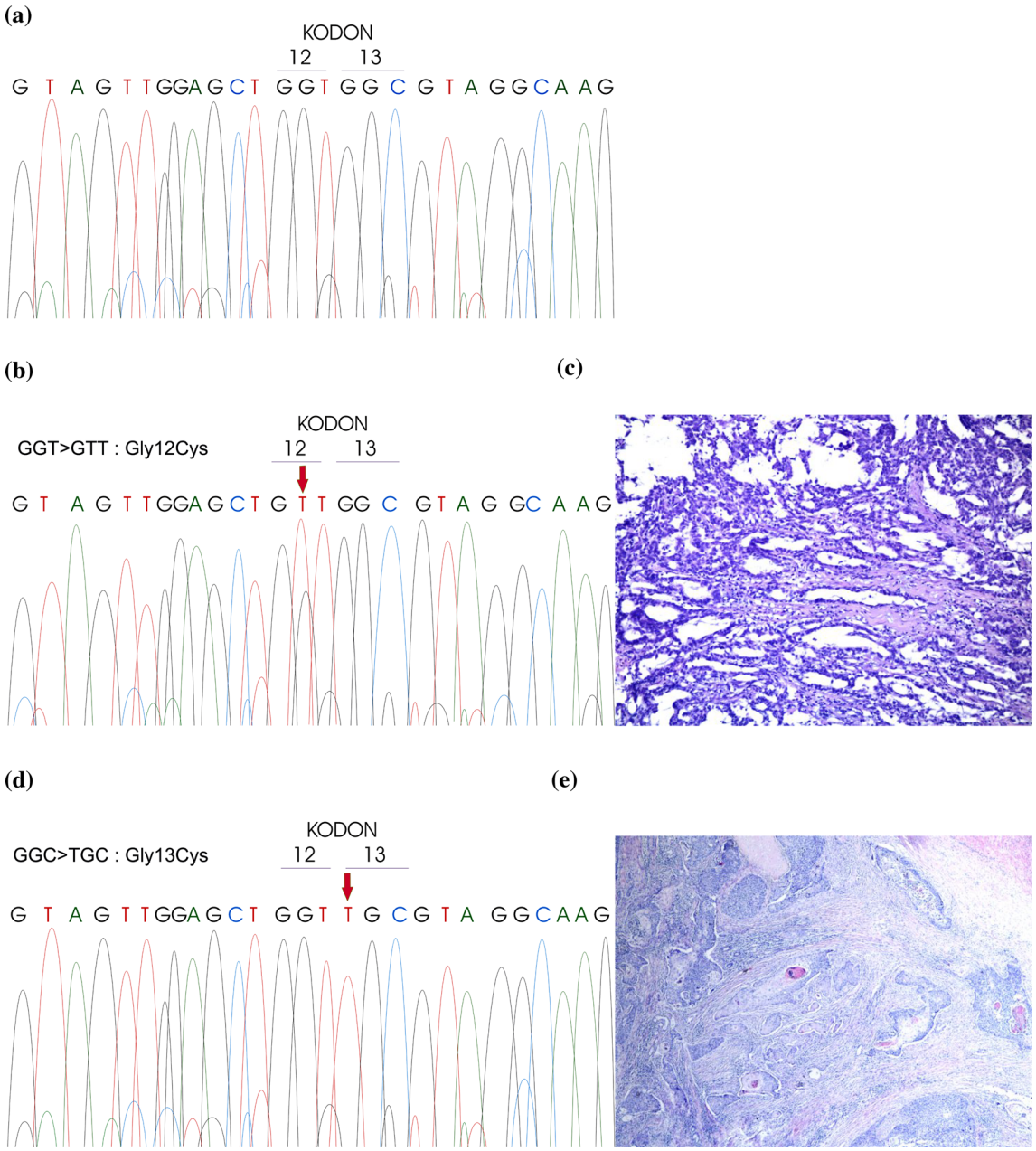

Fig. 1 **a** Wild-type codon 12–13 in *KRAS* gene, **b** *KRAS* codon 12 mutation (GGT>GTT) in case no 4 diagnosed as AC (HEx100) in **c**, and **d** codon 13 mutation (GGC>TGC) in case no 16 diagnosed as SCC (HEx40) in **e**

usually occur in AC, and rarely in SCC, and not in small cell lung cancer [6, 23]. In our study, the rates of *KRAS* mutation were 24 % in NSCLC and 21.4 % in AC as similar in Western countries. However, similarly *EGFR* mutations, it was high rate (27.3 %) in SCC patients in contrast to other studies. In terms of histological types, *KRAS* mutations are associated more with mucinous BAC or goblet cell morphology than non-mucinous BAC [4, 6, 27]. Authors suggested that mucinous differentiation was significantly correlated with presence of *KRAS* mutation and absence of *EGFR* mutation [9, 27]. *KRAS* mutations predominantly occur in White people with a history of smoking [4, 19]. However, Riely et al. [22] found that *KRAS* mutations were not rare (15 %) among never smokers. Although AC of the lung is thought to be associated less with smoking than SCC or small cell carcinoma, the studies have reported the association between *KRAS* mutations and smoking status [6].

Approximately 80 % of *KRAS* mutations occur in codon 12, and predominant mutation is a G to T transversion (70 % of tumors) [6, 24]. In our study, as similarly, most *KRAS* mutations were localized at codon 12 (5/6), and only

one mutation was at codon 13. Most of them were G to T transversion mutation as consistent with other studies. The studies have reported that transversion mutations (G–T or G–C) are associated with smoking history, while transition mutations (G–A) are more common in patients with never smoking history [6, 21, 22]. In our study, four of six patients with *KRAS* mutation (three G>T, one T>C) were ex- or current smokers, while two patients with G>A and G>T mutation were never smokers. *EGFR* mutations are the first molecular aberrations found more frequently among in never smoking patients than smoking patients. In contrast to *KRAS*, its frequency is inversely associated with the smoking dosage [4, 6, 19, 22]. However, *EGFR* mutations have also been detected in patients with a history of smoking, as seen in our study [4, 19]. Authors suggest that some *EGFR* mutations are caused by carcinogen other than those contained in tobacco smoke [4, 6, 19]. On the other hand, *KRAS* mutations are present in those with significant tobacco exposure and are adverse prognostic factors [9, 21].

The studies have reported that the occurrence of *EGFR* and *KRAS* mutations is mutually exclusive in NSCLC [1, 2, 4, 6]. However, there are few reports in the literature that coexistence of *EGFR* and *KRAS* mutations in the same tumor [1, 2, 31, 32]. In our study, we detected both *EGFR* and *KRAS* mutations simultaneously in the three cases (two AC, one SCC). These data suggest that all cases are not always mutually exclusive and some cases may contain *EGFR* and *KRAS* mutations simultaneously. This combined mutation status is important issue since it may have impact on the clinical outcome and future treatment decisions. This particular subgroup of patients may require a different therapy compared to only *EGFR* mutated tumors.

EGFR and *KRAS* are the two most frequently mutated genes in AC of the lung [6]. These assays can be performed on archival FFPE tissue, although fresh tissue is the best for PCR-based tests [9]. Although larger tumor samples obtained from surgery are preferred for molecular analysis, small specimens including bronchial biopsy and needle core biopsies are available for diagnostic tests in most of patients with advanced stage NSCLC, which they may be only material available for genomic analysis. Sufficient results may be obtained from these small lung biopsies [9, 33–37]. Genetic analysis should be done, even if only small biopsy materials are available. Thus, decision of the optimal treatment could be done before any drugs are used [33]. However, Warth et al. [34] have recently showed that histologically estimated tumor concentration of 40 % is necessary for reliable detection of all mutations by Sanger sequencing in lung biopsy materials, although mutations might also be detected in samples with low tumor concentration (<40 %), 10–20 % for exon 19 and 30 % for exon 21. As consistent with this study, in our study, tumor concentration was ranged 20–80 % in all patients.

In this study, approximately one-third of the patients are at operable (stage II–IIIA). These patients are not routinely tested for *EGFR* mutations as they do not receive EGFR-TKI treatment. But it would be useful to perform *EGFR* and *KRAS* gene mutations in this operable subpopulation of lung cancer patients since the recurrence rate is very high even if they had surgery.

In conclusion, in this study, we firstly examined *EGFR* and *KRAS* mutations in small number of Turkish NSCLC patients both in AC and SCC. The frequency and characteristics of *KRAS* gene mutation, especially in ACs, were found quite similar to those seen in the Western countries. On the other hand, the frequency and clustering of *EGFR* mutations were quite similar to those of East Asian rather than the Westerns countries. Furthermore, simultaneously, *EGFR* and *KRAS* mutations were detected in some cases. Interestingly, the mutation rates in both *KRAS* and *EGFR* genes in SCCs were higher than the literature. According to these findings, we can suggest that these mutations should be tested in especially small diagnostic biopsy materials of SCC cases. Thus, further studies including more cases should be performed to verify our results in Turkish NSCLC patients.

Conflict of interest All the authors declare that no conflict of interest.

References

1. Yamamoto H, Toyooka S, Mitsudomi T. Impact of EGFR mutation analysis in non-small cell lung cancer. Lung Cancer. 2009;63:315–21.
2. Cheng L, Zhang S, Alexander R, et al. The landscape of EGFR pathways and personalized management of non-small-cell lung cancer. Future Oncol. 2011;7:519–41.
3. Inamura K, Ninomiya H, Ishikawa Y, et al. Is the epidermal growth factor receptor status in lung cancers reflected in clinicopathologic features? Arch Pathol Lab Med. 2010;134:66–72.
4. Mitsudomi T, Yatabe Y. Epidermal growth factor receptor in relation to tumor development: EGFR gene and cancer. FEBS J. 2010;277:301–8.
5. Pao W, Miller V, Zakowski M, 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. PNAS. 2004;101:13306–11.
6. Suda K, Tomizawa K, Mitsudomi T. Biological and clinical significance of *KRAS* mutations in lung cancer: an oncogenic driver that contrasts with EGFR mutation. Cancer Metastasis Rev. 2010;29:49–60.
7. Lynch TJ, Bell DW, Sordella R, 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;350:2129–39.
8. Paez JG, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science. 2004;304:1497–500.

9. Dacic S. EGFR assays in lung cancer. Adv Anat Pathol. 2008;15:241–7.
10. Lee CK, Brown C, Gralla RJ, et al. Impact of EGFR inhibitor in non-small cell lung cancer on progression-free and overall survival: a meta-analysis. J Natl Cancer Inst. 2013;105:595–605.
11. Garassino MC, Marsoni S, Floriani I. Testing epidermal growth factor receptor mutations in patients with non-small-cell lung cancer to choose chemotherapy: the other side of the coin. J Clin Oncol. 2011;29:3835–7.
12. Tanaka T, Matsuoka M, Sutani A, et al. Frequency of and variables associated with the EGFR mutation and its subtypes. Int J Cancer. 2010;126:651–5.
13. Liu Y, Xu ML, Zhong HH, et al. EGFR mutations are more frequent in well-differentiated than in poor-differentiated lung adenocarcinomas. Pathol Oncol Res. 2008;14:373–9.
14. Bae NC, Chae MH, Lee MH, et al. EGFR, ERBB2, and KRAS mutations in Korean non-small cell lung cancer patients. Cancer Genet Cytogenet. 2007;173:107–13.
15. Li M, Zhang Q, Liu L, et al. The different clinical significance of EGFR mutations in exon 19 and 21 in non-small cell lung cancer patients of China. Neoplasma. 2011;58:74–81.
16. Blons H, Cote JF, Le Corre D, et al. Epidermal growth factor receptor mutation in lung cancer are linked to bronchioloalveolar differentiation. Am J Surg Pathol. 2006;30:1309–15.
17. Na II, Rho JK, Choi YJ, et al. Clinical features reflect exon sites of EGFR mutations in patients with resected non-small-cell lung cancer. J Korean Med Sci. 2007;22:393–9.
18. Brandao GDA, Brega EF, Spatz A. The role of molecular pathology in non-small-cell lung carcinoma—now and in the future. Curr Oncol. 2012;19:S24–32.
19. Mitsudomi T, Yatabe Y. Mutations of the epidermal growth factor receptor gene and related genes as determinants of epidermal growth factor receptor tyrosine kinase inhibitors sensitivity in lung cancer. Cancer Sci. 2007;98:1817–24.
20. Ladanyi M, Pao W. Lung adenocarcinoma: guiding EGFR-targeted therapy and beyond. Mod Pathol. 2008;21:S16–22.
21. Riely GJ, Marks J, Pao W. KRAS mutations in non-small cell lung cancer. Proc Am Thorac Soc. 2009;6:201–5.
22. Riely GJ, Kris MG, Rosenbaum D, et al. Frequency and distinctive spectrum of KRAS mutations in never smokers with lung adenocarcinoma. Clin Cancer Res. 2008;14:5731–4.
23. Wu CC, Hsu HY, Liu HP, et al. Reversed mutation rates of KRAS and EGFR genes in adenocarcinoma of the lung in Taiwan and their implications. Cancer. 2008;113:3199–208.
24. Wang Z, Wu YL, Zhang GC, et al. EGFR/KRAS mutations and gefitinib therapy in Chinese NSCLC patients. Onkologie. 2008; 31:174–8.
25. Massarelli E, Varella-Garcia M, Tang X, 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;13:2890–6.
26. Park SH, Ha SY, Lee JI, et al. Epidermal growth factor receptor mutations and the clinical outcome in male smokers with squamous cell carcinoma of lung. J Korean Med Sci. 2009;24:448–52.
27. Finberg KE, Sequist LV, Joshi VA, et al. Mucinous differentiation correlates with absence of EGFR mutation and presence of KRAS mutation in lung adenocarcinomas with bronchioloalveolar features. J Mol Diagn. 2007;9:320–6.
28. Remon J, Morán T, Majem M, et al. Acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in EGFR-mutant non-small cell lung cancer: a new era begins. Cancer Treat Rev. 2014;40:93–101.
29. Guan JL, Zhong WZ, An SJ, et al. KRAS mutation in patients with lung cancer: a predictor for poor prognosis but not for EGFR-TKIs or chemotherapy. Ann Surg Oncol. 2013;20:1381–8.
30. Linardou H, Dahabreh IJ, Kanaloupiti D, et al. Assessment of somatic k-RAS mutations as a mechanism associated with resistance to EGFR-targeted agents: a systematic review and meta-analysis of studies in advanced non-small-cell lung cancer and metastatic colorectal cancer. Lancet Oncol. 2008;9:962–72.
31. Schmid K, Oehl N, Wrba F, et al. EGFR/KRAS/BRAF mutations in primary lung adenocarcinomas and corresponding locoregional lymph node metastases. Clin Cancer Res. 2009;15:4554–60.
32. Jürgens J, Engel-Riedel W, Prickartz A, et al. Combined point mutation in KRAS or EGFR genes and EML4-ALK translocation in lung cancer patients. Future Oncol. 2014;10:529–32.
33. Masago K, Fujita S, Mio T, et al. Accuracy of epidermal growth factor receptor gene mutation analysis by direct sequencing method based on small biopsy specimens from patients with non-small cell lung cancer: analysis of results in 19 patients. Int J Clin Oncol. 2008;13:442–6.
34. Warth A, Penzel R, Brandt R, et al. Optimized algorithm for Sanger sequencing-based EGFR mutation analyses in NSCLC biopsies. Virchows Arch. 2012;460:407–14.
35. Solomon SB, Zakowski MF, Pao W, et al. Core needle lung biopsy specimens: adequacy for EGFR and KRAS mutational analysis. Am J Roentgenol (AJR). 2010;194:266–9.
36. Lim EH, Zhang SL, Yu K, et al. An alternative approach to determining therapeutic choices in advanced non-small cell lung carcinoma (NSCLC): maximizing the diagnostic procedure and the use of low-volume lung biopsies. J Thorac Oncol. 2007;2: 387–96.
37. Cheung YC, Chang JW, Hsieh JJ, et al. Adequacy and complications of computed tomography-guided core needle biopsy on non-small cell lung cancers for epidermal growth factor receptor mutations demonstration: 18-gauge or 20-gauge biopsy needle. Lung Cancer. 2010;67:166–9.