ORIGINAL ARTICLES

Clinical Investigations

EPIDERMAL GROWTH FACTOR RECEPTOR ACTIVATING MUTATIONS IN SQUAMOUS HISTOLOGY OF LUNG CANCER PATIENTS OF SOUTHERN BULGARIA

Silvia N. Genova^{1*}, Stoian N. Bichev², Vladimir G. Kanarev³

¹Department of General and Clinical Pathology and Forensic Medicine, Faculty of Medicine, Medical University, Plovdiv, ²National Genetics Laboratory, Maichin dom University Obstetrics and Gynecology Hospital, Sofia, ³Department of Medical Oncology and Radiotherapy, Tsaritsa Joanna University Hospital ISUL, Sofia, Bulgaria

АКТИВИРУЮЩИЕ МУТАЦИИ В РЕЦЕПТОРЕ ЭПИДЕРМАЛЬНОГО ФАКТОРА РОСТА В ПЛОСКОКЛЕТОЧНЫХ КАРЦИНОМАХ ЛЁГКИХ У ПАЦИЕНТОВ ИЗ ЮЖНОЙ БОЛГАРИИ

Силвия Н. Генова^{1*}, Стоян Н. Бичев², Владимир Г. Канарев³

¹Кафедра общей и клинической патологии и судебной медицины, Факультет медицины, Медицинский университет, Пловдив, ²Национальная лаборатория генетики, Университетская больница, СБАЛАГ „Майчин дом“ - София, ³Клиника медицинской онкологии и лучевой терапии, УМБАЛ „Царица Йоанна - ИСУЛ“ - София, Болгария

ABSTRACT

There is only limited data on the prevalence of epidermal growth factor receptor (EGFR) activating mutations in squamous cell carcinomas and adenosquamous carcinomas of the lung in patients of the Southern Bulgarian region and the efficacy of EGFR tyrosine kinase inhibitors. **Aim:** Previous reports for Bulgarian population showed high incidence of EGFR mutations in the squamous cell carcinomas, so we set the goal to investigate their frequency in Southern Bulgaria, after precise immunohistochemical verification of lung cancers. **MATERIALS AND METHODS:** Two hundred and thirty-six lung carcinomas were included in this prospective study. All biopsies were initially analysed with p63, TTF1, Napsin A, CK7, CK34βE12, synaptophysin, CK20 and CDX2. Two hundred and twenty-five non-small cell lung carcinomas were studied with real-time PCR technology to assess the status of the EGFR gene. **RESULTS:** We detected 132 adenocarcinomas (58.7%), 89 squamous cell carcinomas (39.2%), 4 adenosquamous carcinomas (1.8%), 9 large cell neuroendocrine carcinomas (3.8%) and 2 metastatic colorectal adenocarcinomas (0.8%). Activating mutations in the EGF receptor had 3 out of 89 squamous cell carcinomas (3.37%). We have established mutations in L858R, deletion in exon 19 and rare mutation in S7681. One out of four adenosquamous carcinomas had a point mutation in the L858R (25%). **CONCLUSIONS:** The frequency of EGFR mutations we found in lung squamous cell carcinomas in a Southern Bulgarian region is lower than that in European countries. Ethnic diversity in the region does not play role of an independent predictive factor in terms of mutation frequency.

Key words: EGFR gene, lung, squamous cell carcinoma, adenosquamous carcinoma

Folia Medica 2015;57(3&4):191-199

Copyright © 2015 Medical University, Plovdiv

РЕЗЮМЕ

Почти отсутствуют данные о частоте данных мутаций при плоскоклеточных и аденосквамозных карциномах у пациентов из Южной Болгарии, а также об эффективности терапии ингибиторами эпидермального фактора роста. **Цель:** Так как предыдущие исследования болгарских популяций показывают высокую частоту мутаций эпидермального фактора роста при плоскоклеточных карциномах, мы поставили своей задачей исследование данной частоты в популяциях из Южной Болгарии после точной иммунной гистологической верификации опухоли лёгких. **Материалы и методы:** В проспективное исследование было включено 236 карциномов лёгких. Все биопсии были протестированы p63 TTF1, Napsin A, CK7, CK34βE12, Synaptophysin, CK20 и CDX2. Для оценки состояния гена эпидермального фактора роста было исследовано 225 случаев немелкоклеточной карциномы лёгких при помощи полимеразной цепной

Article's history: Received: 11 Aug 2015; Received in a revised form: 29 Feb 2016 Accepted: 02 March 2016

*Correspondence and reprint request to: S. Genova, Department of General and Clinical Pathology and Forensic Medicine, Faculty of Medicine, Medical University, Plovdiv; E-mail: sylvia_genova@abv.bg; Tel.: +359 888 811 485
15A Vassil Aprilov Blv., 4002 Plovdiv, Bulgaria

реакции в реальном времени. **Результаты:** Установлены 132 аденокарциномы (58.7%), 89 плоскоклеточных карцином (39.2%), 4 аденосквамозные карциномы (1.8%), 9 крупноклеточных нейроэндокринных карцином (3.8%) и 2 метастазные колоректальные аденокарциномы (0.8%). Наличие активирующих мутаций в рецепторе эпидермального фактора роста установлено в трёх из 89 плоскоклеточных карцином (3.37 %). Установлены мутации в L858R, делеция в 19 экзоне и редкая мутация в S7681. На каждые четыре аденосквамозные карциномы приходится одна с точечной мутацией в L858R (25 %). **Выводы:** Наблюдаемая частота мутаций эпидермального фактора роста плоскоклеточных карцином лёгких в Южной Болгарии ниже по сравнению с другими европейскими странами. Этническое разнообразие в регионе не играет роли независимого фактора, предсказывающего частоту подобных мутаций.

Ключевые слова: ген рецептора эпидермального фактора роста, лёгкие, плоскоклеточная карцинома, аденосквамозная карцинома

Folia Medica 2015;57(3&4):191-199

© 2015 Все права защищены. Медицинский университет, Пловдив

INTRODUCTION

Non-small cell lung carcinomas (NSCLC) are approximately 85% of newly diagnosed cancers of the lung, with two major histological subtypes: approximately 50% are adenocarcinomas (AC) and 30% squamous cell carcinomas (SCC). Lung SCC is closely associated with tobacco smoking (up to 400 000 deaths per year, according to the WHO statistics).¹ The discovery of the so-called “driver mutations”, such as the epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK) rearrangements has led to remarkable improvement in the personalized therapy of lung adenocarcinoma.^{2,3} Efforts at conservative therapy are aimed primarily at ACs, since there is currently no personalized therapy for the SCCs.

Whole exon/genome sequencing of lung SCC allows identification of several potential mutations in the signaling pathways. These are FGFR1 amplifications, PI3K/ACT mutations, PTEN mutations/deletions, PDGFRA mutations, as well as DDR2 mutations. The transition to personalized therapy can be a step forward in discovering these potential biomarkers for the treatment of lung SCC.^{3,4} However, no such available molecular target exists for lung carcinomas with squamous cell histology at present. Therapies with bevacizumab, pemetrexed and EGFR tyrosine-kinase inhibitors have been developed for lung adenocarcinomas, but are contraindicated or largely ineffective in lung SCC. Patients with lung SCC are treated with one-size-fits-all platinum-based chemotherapy.^{5,6}

Adenosquamous (ADSQ) lung carcinoma contains components of both, squamous cell carcinoma and adenocarcinoma, the proportion of each of them being no less than 10% of the tumor mass. It is a rare subtype, accounting for 0.4 to 4% of all

NSCLC. Because of its rarity, clinicopathological characteristics of ADSQ carcinomas have not been adequately studied, and its pathogenesis is still unclear. The general prognosis of this tumor is worse than that of AC and SCC.⁷

In recent years in two large prospective studies of NSCLC in Bulgaria, Damyanov D. et al. reported 9.4% incidence of EGFR mutations in 752 patients with lung cancer.^{8,9} In the group of activating EGFR mutations positive patients (n = 71), the prevalence of adenocarcinomas was 59.7% (43/71). It is worth noting that EGFR mutations occurred relatively frequently in squamous cell carcinoma patients (33.9%, 24/71). These results, however, were reported without immunohistochemical analysis. This is perhaps the reason why the mutations found in squamous cell carcinomas are far more frequent than those reported by other authors whose studies have been conducted on an adequate number of cases.^{10,11,19}

The considerable discrepancy in the reported data could be due to differences in the methodology of performing the mutation analysis, but could be also a result of genetic differences between eastern patient populations and other populations.⁷ Such ethnic diversity can be found, to a certain extent, in Southern Bulgaria, which makes it necessary to compare these data with data from other regions of the country.

AIM

The aim of the present study was a detailed histological and immunohistochemical categorization of non-small cell lung carcinomas with EGFR mutations in patients in Southern Bulgaria having in mind the ethnic diversity in the region.

MATERIALS AND METHODS

BIOPSY SELECTION

Between 2012 and 2014, we performed a prospective study of 236 newly found tumors in the lungs of patients from regions in Southern Bulgaria (45.36% Bulgarians and 54.65% Turks). The male patients were 201, and the female – 35 (a proportion ratio of 5.7:1, age range - 28 to 84 years). The tumors had the greatest incidence in the age range of 61-70 years - 115 cases (48.7%).

Carcinomas which demonstrated neuroendocrine differentiation (synaptophysin positive) or were metastatic colorectal adenocarcinomas (CK20 and CDX2 positive), a total of 11 cases, were not subjected to a genetic study. The biopsy material was analyzed immunohistochemically in the laboratory of the Medical University in Plovdiv using two markers - p63 and TTF1, and PAS reaction for mucin. Double negative tumors were in addition verified with Napsin A, CK7, CK34βE12, synaptophysin, CK20 and CDX2.

DNA EXTRACTION, EGFR GENE MUTATION ANALYSIS

Molecular analysis was performed at the National Genetic Laboratory, Sofia. Two hundred and twenty-five biopsy samples of primary lung carcinomas were studied for EGFR mutations. Tested samples were taken from paraffin-embedded, surgical and endoscopic biopsies. For DNA analysis we selected only the cases with more than 50% tumour cells in the sections, employing 10 cuts of 5 μm. For the EGFR gene mutation analysis we used the Scorpions Amplification Refractory Mutation System (SARMS) technology (Qiagen). The ARMS PCR technology combined with the Scorpions detection

technology provides fast and reliable technology for detection of mutations in FFPET. This method is more sensitive and specific for detection of known mutations than dideoxy sequencing technology. The specifically designed EGFR RGQ PCR kit allows the detection of 29 known recurrent mutations (including the inhibitory mutation T790M) in exons 18-21 of the EGFR gene, including 19 deletions in exon 19 (does not distinguish between them), T790M in exon 20, L858R in exon 21, L861Q in exon 21, G719X in exon 18 (G719S, G719A or G719C, but does not distinguish between them), S768I in exon 20 and 3 insertions in exon 20 (does not distinguish between them). The various reactions were performed according to the manufacturer's instructions and the analyses were carried out for all the samples as described in the kit instruction manual. A Rotor-Gene Q MDx machine was used for performing the PCR reactions.

RESULTS

Out of the 225 cases with NSCLC in patients from Southern Bulgaria, 132 (58.7%) were found to be AC, 89 (39.2%) - SCC and four cases (1.8%) - ADSQ. Thirteen cases (5.77%) were found to be positive for EGFR mutation. Nine (3.99%) of these tumours were classic adenocarcinomas (Table 1). In three patients the mutations involved the L858R region. Four were with deletion in exon 19. Only one positive patients was of ethnic Turkish origin - a female, non-smoker, with adenocarcinoma and rare mutation in L861Q (Table 1, Case 4).

In the group with EGFR mutations in the SCC (3/89, 3.37%) the patients were predominantly male, active and former smokers with 30-40 pack years

Table 1. Patient characteristics and EGFR gene mutations in lung adenocarcinomas

No	Age yrs	Gender	Smoking history	Mutations in EGFR	Histological subtype
1	77	male	Former, 40 PY	L858R	Adenocarcinoma
2	58	male	Current, 35 PY	L858R	Adenocarcinoma
3	67	male	Current, 40 PY	L858R	Adenocarcinoma
4	60	female	No	L861Q	Adenocarcinoma
5	58	female	No	Deletion in exon 19	Adenocarcinoma
6	58	female	No	Deletion in exon 19	BAC (Lepidic AC)
7	69	female	No	Deletion in exon 19	Papillary AC
8	62	male	Former, 40 PY	Deletion in exon 19	Papillary AC
9	55	male	Current, 40 PY	Insertion in exon 20	Mucinous Papillary AC

Legend: PY- pack years.

Table 2. Patient characteristics and EGFR gene mutations in SCC and ADSQ carcinomas in Southern Bulgaria

NO	Age yrs	Gen- der	Smoking history	Histology	EGFR mutations	Treatment with TKI-PFS	IHC	
							Positive +	Negative -
1	65	male	Current, 40 PY	SCC	L858R	Progression after 4 mos	P63	TTF1
2	64	male	Former, 25 PY	SCC	Deletion exon 19	19 mos PFS [†]	P63	TTF1
3	49	male	Current, 30 PY	SCC	S7681	no [†]	P63 CK34βE12 CK7	Napsin A TTF1
4	75	female	no	ADSQ	L858R	no	Napsin A TTF1 CK34βE12 CK7	P63

Legend: SCC - squamous cell carcinoma; ADSQ - adenosquamous carcinoma; PY - pack years; mos – months; PFS – progression free survival; † - lethal outcome.

(PY), (Table 2). Three cases were squamous cell carcinomas and one - adenosquamous cell carcinoma (1.78% of all NSCLCs in the study group). Only one case (Case 4) with squamous cell component had a profile similar to the profiles typical for the EGFR-mutation carcinomas (a female, non-smoker, with malignant adenomatous component), but it was a case of adenosquamous carcinoma. There were also three cases of ADSQ without EGFR-mutations (1/4, 25%). None of the patients with squamous cell component, positive for mutations, were of Turkish ethnic origin (Table 2).

CASE STUDY

Case 1. A 65-year-old male presented with centrally located tumour in the right lung. The tumour involved the visceral and parietal pleura, with metastases in mediastinal lymph nodes (IIIb clinical stage). The patient was a current smoker with a smoking history of 40 pack-years. Endoscopic biopsy findings indicated moderately differentiated squamous cell carcinoma (G2), with diffuse strong expression of p63 (3+) and TTF1 (-) negative signal. Genetic study provides mutation in L858R. The patient received TKI therapy, but after tumour progression at month 4 of treatment, the therapy was discontinued and was replaced with standard conservative chemotherapy.

Case 2. A 64-year-old male, former smoker (25 PY). Tumour mass was located in the right lung with distant bone metastases (stage IV). Bronchoscopic biopsy found a moderately differentiated SCC (G2), positive for EGFR mutations in exon

19. The patient had responded very well to TKI therapy for 19 months with partial reduction of lung cancer (Figs 1A and 1B) and partial reduction of bone metastases (Figs 1C and 1D). After tumour progression at month 19 of treatment, the therapy was discontinued and was replaced with standard conservative chemotherapy. The patient died after progression of the disease.

Case 3. A 49-year-old male, current smoker, had a tumour mass measuring 4 by 3 cm in the right middle pulmonary lobe. The patient was subjected to right lobectomy: the finding was poorly differentiated (G3) squamous cell carcinoma. Immunohistochemical study: diffuse expression of p63 (3+); CK34βE12 (3+); CK7 (3+) and negative markers for TTF1 (-); Napsin A (-). The tumor was positive for EGFR in S7681. The patient died six weeks after surgery before receiving target therapy or chemotherapy.

Case 4. A female, 75 years old, non-smoker, with central tumour mass located in the right lung, measuring 4 by 2.5 cm. This patient underwent right pneumonectomy. Histological study: a moderately differentiated (G2) adenosquamous carcinoma, the adeno-component was mucin-producing. The squamous cell component was part of the glands and histologically it appeared as squamous cell metaplasia, having the characteristics of adenocarcinoma. These regions were negative for p63 (-). The adenomatous component displayed a diffuse high expression of Napsin A (3+); TTF1 (3+); CK7 (3+); CK 34βE12 (3+) (Fig. 2). The genetic study was positive for EGF receptor mutation in L858R.

Figure 1. Chest computed tomography showed primary tumor in the right lung (A) and its partial remission after 19 months of TKI treatment (B). Gamma-camera bone scintigraphy with ^{99m}Tc -MDP (C) and partial remission after 6 months of TKI treatment (D).

The patient was staged as IIa clinical stage. In the absence of carcinoma metastases, patient was subjected to a course of standard platinum-based chemotherapy, showing stable condition without disease progression.

DISCUSSION

EGFR and KRAS mutations are oncogenic aberrations that are highly prevalent in adenocarcinomas but extremely rare in squamous cell carcinomas. Squamous cell carcinomas more often display changes in

the signalling pathways of PI3K/AKT and tyrosine-kinase receptors, including EGFR amplification, as well as BRAF mutations or FGFR amplification or mutations (in 69% of samples). Loss-of-function mutations and mutations in HLA-A class I major histocompatibility gene have been found in 3% of tumours, which suggests a possible role of genotypic selection of patients for immunotherapies such as anti-programmed death 1 (PD1) and anti-cytotoxic T-lymphocytic antigen 4 (CTLA4).¹²

ADSQ lung carcinomas are rare histological

A

B

C

D

E

F

Figure 2. IHC; Intensive positive, cytoplasmic markers in ADSQ, (A, C, E), Original magnification; A) Napsin A, x20; B) TTF1, nuclear staining, x40; C) CK7, x20; D) P63 negative staining in squamous component, x40; E) CK34βE12, x10; F) ADSQ carcinoma, glandular structures with squamous metaplasia H.E. x40.

subtypes of non-small cell lung carcinomas (only 2.1 – 3.4% of these cancers). It has a worse prognosis than that of classic adenocarcinomas and that of “pure” squamous cell carcinomas. Although the etiology of this cancers is still unknown, EGFR mutations have also been detected in this subtype with prevalence similar to that of AC.¹⁸

T. Shiozawa et al., H. Sasaki et al., S. Toyooka et al., S. Kang et al., and XL Jia et al. detected EGFR mutations between 15%-44% among Korean, Japanese and Chinese populations.^{7, 13-16} Wang R et al. found a high prevalence of these mutations in a sample of population (31.6%, 24/76 and 38%, 21/55, respectively).¹⁷ The discrepancy could be due to differences in the methodology used in mutation analysis but could also be a result of genetic differences between Eastern populations and other populations.⁷ We are interested in a recent pilot study among the Turkish population, where EGFR mutations in SCC patients were found in more than half of the studied tumours (54.5%, 6 out of 11), which is consistent with the prevalence rate reported in Asian populations.¹⁸ (Table 3).

Miyamae Y, et al. examined 89 squamous cell lung carcinomas for EGFR mutations in exons 19 and 21. The initial prevalence was 5.6%, all with deletion in exon 19. Immunohistochemical analysis with p63, TTF-1 and staining for mucin using Al-

cian Blue demonstrated an adenomatous component in two of the tumours. Thus, the percentage of positive SCC was reduced to 3.4% (3/87). The researchers found a small yet significant number of positive mutations in squamous cell carcinomas, which justified the genotyping of all NSCLC, making the diagnostic process even more accurate and giving chance to such patients to be treated with TKI therapy.¹¹ The EGFR mutations we found in the large sample size SCC are consistent with the data of these authors (3.37%), while the highest positivity rates in ADSQ carcinomas (25%) is not informative, since due to the small number of studied cases. The overall incidence of mutations in all cancers are found to be lower than the mean prevalence in European population and only one patient of a different ethnic origin was found to have activating mutations. In our study ethnic diversity in Southern Bulgaria is not an independent prediction factor for higher mutation frequency of EGFR in SCC and ADSQ carcinomas.

Analysis of the mutations and their correlation with etiologic and morphogenetic factors lead to contradictory conclusions: Sasaki et al. reported that mutational status of EGFR – ADSQ carcinoma significantly correlated with smoking history and gender.¹³ Kang et al. found that EGFR mutations in ADSQ carcinoma patients were more common

Table 3. Summarized data for the frequency of EGFR gene mutations in patients with ADSQ and SCC in different regions

Lung carcinomas	Study	Year	Region	EGFR mutations/contingent	
Adenosquamous cell carcinomas	Toyooka S, et al.,	2006	China	3/11	27%
	Kang SM, et al.,	2007	Korea	11/25	44%
	Sasaki H, et al.,	2007	Japan	4/26	15%
	Jia XL, et al.,	2011	China	21/55	38%
	Shiozawa T, et al.,	2013	Japan	14/59	24%
	Wang R, et al.,	2014	China	24/76	31.6%
	Powrozek T, et al.,	2014	Poland	4/14	28.6%
<i>Results from our studies</i>			<i>Southern Bulgaria</i>	<i>1/4</i>	<i>25%</i>
Squamous cell carcinomas	Miyamae Y, et al.,	2011	Japan	3/87	3.4%
	Li Ying, et al.,	2013	China	17/208	8.0%
	Fiala O, et al.,	2013	Czech Republic	16/223	7.2%
	Bircan S, et al.,	2014	Turkey	6/11	54.5%
	Damyanov D,	2013	Bulgaria	24/71	33.9%
<i>Results from our studies</i>			<i>Southern Bulgaria</i>	<i>3/89</i>	<i>3.37%</i>

in female patients who had never smoked.¹⁵ EGFR mutations occurred more often in mixed carcinomas with lepidic growth pattern.^{20,21}

Although the pathogenesis of ADSQ carcinomas has not yet been elucidated, similar molecular characteristics such as DNA aneuploidy, loss of heterozygosity and genomic alterations of p53 and K-RAS have been found in both adenomatous and squamous cell carcinomatous components.¹⁴ The fact that, in our single observation, the squamous cell component was benign, i.e. it was an adenoacanthoma, is in favour that the two cell lines might derive from a common precursor cell. Under specific effect of these mutations, the progenitor cells could develop into both types of tumour cells. The presence of identical mutations in both tumour cells components could explain the monoclonality of carcinogenesis pathway. This is also supported by recent larger sample studies in which 27-40% of patients with ADSQ carcinomas harboured EGFR-activating mutations.¹⁶ The first expectation of EGFR mutations in Europe is carried out by Powrozek T. et al. (2014), in patients of Polish origin. Activating mutations of EGFR were observed in 28.6% (four out of 14) of ADSQ carcinoma-bearing patients and shows that the percentage of EGFR mutations detected in ADSQ carcinoma patients was comparable to or even higher than the percentage of EGFR mutations reported in adenocarcinoma cases.¹⁹

The three squamous cell carcinomas and the one ADSQ carcinoma, positive for EGFR gene mutations, constitute 1.78% of all researched NSCLC patients from Southern Bulgaria. These data agree with the results of other authors who examined all NSCLC for EGFR mutations. Liam CK et al. consider that pure squamous cell lung carcinomas lacking any adenocarcinoma component, EGFR mutations positive, have a prevalence of less than 5%.²⁴

EGFR mutations in exons 19 and 21 are useful molecular markers to predict the response to EGFR-TKI therapy. Patients with EGFR mutations have a response rate of approximately 70%, and an overall survival of 30.5 months. And yet, a recent pooled analysis of published reports showed that the prognosis for lung carcinoma patients harbouring EGFR-sensitive mutations and receiving TKI therapy differs for adenocarcinoma patients and for non-adenocarcinoma patients. The latter group included patients with ADSQ carcinoma.²⁶ The cumulative five-year postoperative survival rate of ADSQ carcinoma patients was found to be ap-

proximately 20%, which makes it worse than that of patients with adenocarcinoma or squamous cell carcinoma (40% for both groups).²² Unlike these authors, Paik P et al.²³ found an equal response to TKI therapy in AC and ADSQ carcinoma patients with activating EGFR mutations, 12 months progression-free survival and a median overall survival of 29 months.

Tokumo M, et al.²¹ found that 69% of the patients with adenocarcinoma responded to TKI therapy, and had a median progression-free survival of 9.8 months, while among the patients with non-adenocarcinomas of the lungs response rate to therapy was 35%, and median progression-free survival - only 3.1 months. The reported two cases with ADSQ carcinomas had a stable response to TKI therapy for three years.²¹

In the present study patients with SCC receiving TKI therapy had a different response rates. In Case 1, the patient was less affected by target therapy and made progression and rapid deterioration after 4 months. The patient in Case 2 was stabilized at 19 months of treatment with tyrosine kinase inhibitors with partial reduction of lung cancer and bone metastases.

CONCLUSION

The EGFR mutations in non-adenocarcinoma patients are one of the negative prediction values for the effect of treatment with EGFR inhibitors of tyrosine kinase (EGFR-TKI).²⁶ However, in some cases the therapy had been effective and patients had a very good response. Good response of therapy depends on the stage of the disease and the clinical performance.

The results of this study support the view of the International Association for the Study of Lung Cancer and the Association for Molecular Pathology that lung cancer patients should not be excluded from genetic study on the basis of clinical characteristics that include ethnicity, smoking history and gender.²⁴ We extend this view with the recommendation that genetic studies should not exclude smokers with SCC or ADSQ carcinomas of the lung, giving them the chance to receive an adequate personalized therapy.

ACKNOWLEDGEMENTS

We would like to extend our sincere gratitude to our supervisor, Assoc. Professor Benyamin Anavi MD, PhD, for his competent guidance and commitment in the preparation of this manuscript.

REFERENCES

1. Jemal A, Bray F, Center MM, et al. Global cancer statistics. *CA Cancer J Clin* 2011;61(2):69-90.
2. Kim HS, Mitsudomi T, Soo RA, Cho BC. Personalized therapy on the horizon for squamous cell carcinoma of the lung. *Lung Cancer* 2013;80(3):249-55.
3. Giaccone G. Epidermal growth factor receptor inhibitors in the treatment of non-small-cell lung cancer. *J Clin Oncol* 2005;23(14):3235-42.
4. Kwak EL, Bang YJ, Camidge DR, Shaw AT, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *N Engl J Med* 2010;363(18):1693-703.
5. Scagliotti GV, Parikh P, von Pawel, J Biesma B, et al. Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naïve patients with advanced-stage non-small-cell lung cancer. *J Clin Oncol* 2008;26(21):3543-51.
6. Johnson DH, Fehrenbacher L, Novotny WF, Herbst RS, et al. Randomized phase II trial comparing bevacizumab plus carboplatin and paclitaxel with carboplatin and paclitaxel alone in previously untreated locally advanced or metastatic non-small-cell lung cancer. *J Clin Oncol* 2004;22(11):2184-91.
7. Shiozawa T, Ishii G, Goto K, Nagai K, et al. Clinicopathological characteristics of EGFR mutated adenosquamous carcinoma of the lung. *Pathol Int* 2013;63(2):77-84.
8. Damjanov D, Koinov K, Naseva E, Bichev St. Frequency of activating EGFR mutations in patients with non-small-cell lung cancer in Bulgaria and treatment with gefitinib. *J Bulg Cancer Society* 2013;9(1):28-32 (Bulgarian).
9. Damjanov D. Gefitinib in the treatment of non-small-cell-lung cancer in patients with EGFR positive mutation status. *J Bulg Cancer Society* 2012;8(1):4-8 (Bulgarian).
10. Fiala O, Pesek M, Finek J, Benesova L, et al. Gene mutations in squamous cell NSCLC: insignificance of EGFR, KRAS and PIK3CA mutations in prediction of EGFR-TKI treatment efficacy. *Anticancer Res* 2013;33(4):1705-11.
11. Miyamae Y, Shimizu K, Hirato J, Araki T, et al. Significance of epidermal growth factor receptor gene mutations in squamous cell lung carcinoma. *Oncol Rep* 2011;25(4):921-8.
12. Baik CS, Pritchard CC, Eaton KD, Chow LQ. EGFR mutations in squamous cell lung cancer in never-smokers. *J Thorac Oncol* 2013;8(1):e6-7.
13. Sasaki H, Endo K, Yukiue H, Kobayashi Y, et al. Mutation of epidermal growth factor receptor gene in adenosquamous carcinoma of the lung. *Lung Cancer* 2007;55(1):129-30.
14. Toyooka S, Yatabe Y, Tokumo M, Ichimura K, et al. Mutations of epidermal growth factor receptor and K-ras genes in adenosquamous carcinoma of the lung. *Int J Cancer* 2006;118(6):1588-90.
15. Kang SM, Kang HJ, Shin JH, Kim H, et al. Identical epidermal growth factor receptor mutations in adenocarcinomatous and squamous cell carcinomatous components of adenosquamous carcinoma of the lung. *Cancer* 2007;109(3):581-7.
16. Jia XL, Chen G. EGFR and KRAS mutations in Chinese patients with adenosquamous carcinoma of the lung. *Lung Cancer* 2011;74(3):396-400.
17. Wang R, Pan Y, Li C, Zhang H, et al. Analysis of major known driver mutations and prognosis in resected adenosquamous lung carcinomas. *J Thorac Oncol* 2014;9(6):760-8.
18. Bircan S, Baloglu H, Kucukodaci Z, Bircan A. EGFR and KRAS mutations in Turkish non-small cell lung cancer patients: a pilot study. *Med Oncol* 2014;31(8):87.
19. Powrózek T, Krawczyk P, Ramlau R, Sura S, et al. EGFR gene mutations in patients with adenosquamous lung carcinoma. *Asia Pac J Clin Oncol* 2014;10(4):340-5.
20. Blons H, Côté JF, Le Corre D, Riquet M, et al. Epidermal growth factor receptor mutation in lung cancer are linked to bronchioloalveolar differentiation. *Am J Surg Pathol* 2006;30(10):1309-15.
21. Tokumo M, Toyooka S, Kiura K, Shigematsu H, 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.
22. Iwanaga K, Sueoka-Aragane N, Nakamura T, et al. The long-term survival of a patient with adenosquamous lung carcinoma harboring EGFR-activating mutations who was treated with gefitinib. *Intern Med* 2012;51(19):2771-4.
23. Paik PK, Varghese AM, Sima CS, Moreira AL, et al. Response to erlotinib in patients with EGFR mutant advanced non-small cell lung cancers with a squamous or squamous like component. *Intern Med* 2012;51(19):2771-4.
24. Liam CK, Leow HR, Pang YK. EGFR mutation testing for squamous cell lung carcinoma. *Letters to the Editor. J Thorac Oncol* 2013;8(12):e114.
25. Maemondo M, Inoue A, Kobayashi K, Sugawara S, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N Engl J Med* 2010;362(25):2380-8.
26. Shukuya T, Takahashi T, Kaira R, Ono A, et al. Efficacy of gefitinib for non-adenocarcinoma non-small-cell lung cancer patients harboring epidermal growth factor receptor mutations: a pooled analysis of published reports. *N Engl J Med* 2010;362(25):2380-8.