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Oncogenic driver mutations in Vietnamese patients with lung adenocarcinoma

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ABSTRACT

Mutation profiles of oncogenes play essential roles in cancer therapy, but data on the prevalence of lung cancer oncogenic driver mutations in Vietnamese patients are limited. This study aims to evaluate the mutation status of *EGFR*, *ALK*, *ROS1*, *RET*, and *MET* genes and analyze the association of gene mutations with clinicopathological characteristics of lung adenocarcinoma. A total of 179 tumors were collected from lung adenocarcinoma patients. The mutation frequencies of *EGFR*, *ALK*, *ROS1*, *RET* and *MET* genes were 44.6, 7.9, 3.0, 3.0 and 2.0%, respectively. *EGFR*, *ALK*, *ROS1* and *RET* alterations tended to be higher in females. Moreover, rearrangements of *ALK*, *ROS* and *RET* were more prevalent in younger lung adenocarcinoma but skipping at exon 14 MET was more frequent in male and older patients. Stages III and IV seem to accumulate more *ALK*, *RET* and *MET* abnormalities. These findings identified the variation of frequencies of Vietnamese lung adenocarcinoma with different clinicopathological characteristics and established the pioneer data for oncogenic diver mutation of lung cancer in Vietnam.

INTRODUCTION

Lung cancer is the most frequent malignancy and the leading cause of cancer-related mortality worldwide, with non-small cell lung cancer (NSCLC) being the most popular subtype, accounting for approximately 80-85% of all diagnosed cases [1]. The majority of lung cancer patients were diagnosed at advanced stages with poor prognoses [2]. Genetic alteration testing for epidermal growth factor receptor gene (EGFR) and receptor tyrosine kinase (ALK) has become the standard evidence for targeted therapy of advanced NSCLC [3]. In addition to *EGFR* and *ALK*, NSCLC patients also carrying c-ROS oncogene 1 (ROS1) rearrangement were indicated to respond to a different TKI drug crizotinib [4,5]. Rearranged during transfection (RET) rearrangement NSCLC patients can be treated with a combination of RET inhibitors such as cabozantinib, vandetanib and lenvatinib [6,7]. Exon 14 skipping testing for MET proto-oncogene (MET) has also been recommended due to its proven impact on the clinical outcomes of NSCLC patients [8]. Although several researches have already evaluated the frequencies of these genetic alterations in NSCLC patients from many countries, data on the prevalence of oncogenic driver mutations in the Vietnam population are limited [9–11]. Previous studies on Vietnamese patient have focused on *EGFR* mutation and *ALK* rearrangement, lacking comprehensive research for oncogenic driver alterations in non-small cell lung cancer. Our research aims to 1) analyze the frequencies of *EGFR* mutation and *ALK*, *ROS1*, *RET* and *MET* gene rearrangements in a representative cohort of Vietnamese patients with lung adenocarcinoma; and 2) assess the correlation of molecular alterations with clinicopathological patient characteristics.

MATERIALS AND METHODS

Patients and specimens

A total of 179 tumors were collected from incident lung adenocarcinoma patients diagnosed at National Cancer Hospital K, Vietnam, from 2021 to 2022. The agreement informed consent was collected for all patients, and the research was permitted by the Institute for Genomic Research (IGR), Vietnam Academy of Science and Technology (VAST) with number: 02-2022/NCHG-HDDD. The patients have not undergone any chemotherapy, targeted therapy and/or radiotherapy before enrolling in the research. The guidelines of the American Joint Committee on Cancer were applied to analyze the histopathological subtype of the resected specimens. The clinicopathological characteristics of the cohort included gender, age of diagnosis, smoking status, tumor site and disease stage.

Genomic DNA and RNA extraction

The specimen containing at least 30% cancer cells were subjected to genomic DNA and RNA extractions using two commercial products: QIAamp DNA FFPE Tissue Kit and QIAamp RNA FFPE Tissue Kit (Qiagen, Valencia, CA, USA). The quality and quantity of the DNA/RNA obtained were checked using the Bio μLite Drop spectrophotometer (BioDrop, Cambridge, England).

Genetic alteration analysis

Twenty-nine mutations belonging to exon 18-21 of the EGFR tyrosine kinase domain were detected using Therascreen EGFR RGQ PCR kit (Qiagen, Valencia, CA, USA). Analysis alteration of *ALK*, *ROS1*, *RET* and *MET* genes were performed by EasyPGX® ready ALK/ ROS1/ RET/ MET kit simultaneously (Diatech Pharmacogenetics, Via Ignazio Silone, Jesi, Italy).

Statistical analysis

All statistical analyses were performed using SPSS Statistics software (version 20.0, IBM, Ehningen, Germany). Descriptive statistics established the patient clinicopathological characteristics of the study cohort. The results of genetic alterations were described as frequencies and percentages for categorical variables. Associations between genetic abnormality status and patient characteristics were investigated using the chi-square test or Fisher exact test. P-values < 0.05 were considered to be statistically significant.

RESULTS

Characteristics of lung cancer patients

Clinicopathological characteristics of 179 patients, including 120 males and 59 females, were summarized in Table 1. At the diagnostic time, the median age was 60 (from 28 to 85 years). In terms of tumor location, 136 samples were collected from primary tumors, 43 of which were obtained from metastatic sites such as the brain, liver, and lymph nodes. Most cigarette smokers (72/76 smoking patients) were male, while only four females used to smoke. One hundred fifty samples were collected from patients at advanced stages (64 at stages III and 86 at stages IV), and 29 of these were from early stages disease.

Table 1. Clinicopathological characteristics of lung cancer patients.

Clinicopathological characteristics		N	%
		179	
Age			
	≤ 60	80	44.7
	> 60	99	53.3
Gender			
	Male	120	67.0
	Female	59	33.0
Tumor site			
	Primary	136	76.0
	Metastasis	43	24.0
Smocking status			
	Yes	76	42.5
	No	103	57.5
Stages			
	I-II	29	16.2
	III	64	35.8
	IV	86	48.0

Prevalence of *EGFR, ALK, ROS1, RET* and *MET* genes in the lung cancer patients

The alteration status of *EGFR, ALK, ROS1, RET* and *MET* genes were examined in 179 cases with adenocarcinoma lung cancer (Figure 1A). A total of 73 *EGFR* mutations were detected in 179 (40.8%) patients. Exon 19 deletions and exon 21 L858R missense mutations were the most common *EGFR* mutations (data not shown). The distribution was shown a predominance of *EGFR* mutations in the female group compared to the male (54.2% vs. 34.2%). Moreover, *EGFR* mutation was higher in non-smokers (48.5% vs. 30.0%) and primary tumors (44.9% vs. 27.9%) (Figure 1B). No statistically significant differences were found between *EGFR* mutated and *EGFR* wild-type tumors with respect to the age of patients (Table 2).

The prevalence of alteration in *ALK, ROS1, RET* and *MET* were 10.1%, 4.8%, 2.8%, and 2.2%, respectively. *ALK* rearrangement did not exhibit a significant association with clinicopathological parameters such as gender, age, and smoking status of patients, but *ALK* rearrangement was more common in metastasis tumors (18.6% vs. 7.4%) and patients at stage IV (15.1% vs. 7.8 and 0.0% with p=0.049). The abnormalities of *ROS1, RET* and *MET* rarely occurred in lung adenocarcinoma (Figure 1B). There is no significant association between *ROS1, RET* and *MET* alterations and patient characteristics. *ROS1* rearrangement seemed to be more popular in metastasis tumors and non-smokers but *RET* and *MET* alteration was observed to be slightly higher in the smoker and late-stage patients (Table 2).

Table 2. Associations between genetic abnormality status and patient characteristics.

			***EGFR* mutation**			***ALK* mutation**			***ROS1* mutation**			***RET* mutation**			***MET* mutation**		
			Yes	%	*p-value*	Yes	%	*p-value*	Yes	%	*p-value*	Yes	%	*p-value*	Yes	%	*p-value*
		179	73	40.8		18	10.1		8	4.5		5	2.8		4	2.2	
Age					*0.267*			*0.328*			*0.470*			*1.000*			*0.629*
	≤ 60	80	29	36.2		10	*12.5*		3	6.2		2	2.5		1	1.2	
	> 60	99	44	44.4		8	*8.1*		5	3.0		3	3.0		3	3.0	
Gender					*0.010*			*0.972*			*0.117*			*1.000*			*1.000*
	Male	120	41	34.2		12	10.0		3	2.5		4	3.3		3	2.5	
	Female	59	32	54.2		6	10.2		5	8.5		1	1.7		1	1.7	
Tumor site					*0.049*			*0.032*			*0.096*			*1.000*			*0.244*
	Primary	136	61	44.9		10	7.4		4	2.9		4	2.9		2	1.5	
	Metastasis	43	12	27.9		8	18.6		4	9.3		1	2.3		2	3.7	
Smocking status					*0.014*			*0.409*			*0.114*			*0.165*			*0.313*
	Yes	76	23	30.3		6	7.9		1	1.3		4	5.3		3	3.9	
	No	103	50	48.5		12	11.7		7	6.8		1	1.0		1	1.0	
Stages					0.151			0.049			0.959			0.630			
	I-II	29	16	55.2		0	0.0		1	3.4		0	0.0		0	0.0	0.394
	III	64	27	42.2		5	7.8		3	4.7		2	3.1		1	1.6	
	IV	86	30	34.9		13	15.1		4	4.7		3	3.5		3	3.5	

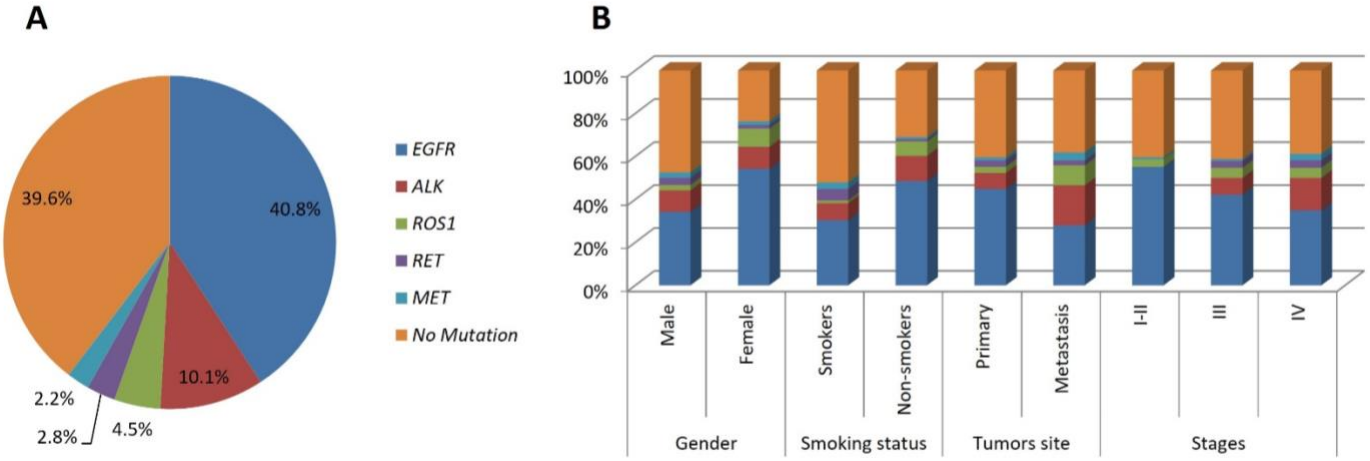

Figure 1. Oncogenic driver mutations in Vietnamese patients with lung adenocarcinoma. Frequency of oncogenic alterations for (A) the overall, (B) each cohort subdivided by patient characteristics such as gender, smoking status, tumor size and stages of tumors.

DISCUSSION

EGFR (also known as HER1) is a member of the human epidermal growth factor family transmembrane receptors including HER1/EGFR, HER2, HER3, and HER4. The dysregulated EGFR protein function or disruptions in any downstream EGFR signaling pathway may result in cell transformation and malignancy [12]. The frequency of *EGFR* mutation is 35 to 50% among the Asian population with lung adenocarcinoma, while this number in Western patients is 10-15% [9–11]. Mutations of *EGFR* are most common in females, non-smokers, young person and Asian ethnic lung cancer patients [8]. About 90% of abnormalities of *EGFR* were exon 19 deletions and L858R substitution in exon 21 [13,14]. In this study, our founding once again confirmed the results of previous research, not only the frequency and distribution of *EGFR* mutation but also the relationships of *EGFR* alterations with clinicopathological characteristics of patients. Notably, *EGFR* mutation was more common in the primary sites than in metastasis locations such as lymph nodes, the brain, and the liver. The differentiation of mutation

frequency may result from secondary genomic changes which induce malignancy cell metastasis.

A high level of ALK expression may lead to activation of a series of cancer signaling pathways: PI3K/AKT, JAK/STAT, and RAS/rapidly accelerated fibrosarcoma (RAF)/ MEK/ERK. Several types of *ALK* alterations have been found in cancer including point mutations, rearrangements, and deletions. In lung cancer, *ALK-EML4* rearrangements are the most common alterations [15,16]. *ALK* rearrangements were detected in approximately 5% of advanced NSCLC. *ALK-EML4* rearrangements were more common in adenocarcinomas, smokers, and late-stage patients. In our research, the *ALK* rearrangements frequency was 10.1% higher than in previous publications. The high mutation rate can result in late diagnostic time for patients of the study cohort. Most Vietnamese lung cancer patients were detected their diseases in III and IV stages with large tumor size, advanced disease, and serious illness. Contrary to *EGFR* mutation, we found that *ALK* rearrangements often occurred more commonly in metastasis tumors and III-IV stages than primary tumors and I-II stages. The changes of driven mutation frequency in different tumor sites of lung adenocarcinoma are shown to the rapid modifications of cancer becoming challenges for disease therapy.

ROS1 is a receptor tyrosine kinase of the insulin receptor family, located on chromosome 6q22 and encodes a transmembrane. ROS1 rearrangements were identified as an oncogenic driver mutation in NSCLC after discovering in glioblastoma [17]. ROS1 fusions have been recognized with about 20 rearrangement partners in NSCLC, including CD74-ROS1, EZR-ROS1, SDC4-ROS1 and SLC34A2-ROS1...[18]. Similar to other alterations in NSCLC, *ROS1* rearrangement seems to be more common in young patients, non-smokers, adenocarcinomas and the Asian population. [19]. Although the limitation of patient number in this study, the *ROS1* rearrangements in Vietnamese lung adenocarcinoma like to be higher in younger, female, metastasis tumors and never-smoker patients. Furthermore, we also found that most of the *ROS1* fusions in our research were determined in exon 34, which belongs to the transmembrane domain (data not shown).

RET gene encodes RET protein, a cell surface tyrosine kinase receptor that lies in chromosome 10 (10q11.2). The combination of RET and ligands may activate serials of pathways such as RAS, MAPK/ERK, PI3K/AKT and JAK/STAT [20]. In NSCLC, *KIF5B* and *CCDC6* are known as the most common partners of *RET* rearrangements [21]. *RET* alterations have been detected in 1-2% of younger and never-smoker adenocarcinoma lung cancer patients [20]. In the total of 179 Vietnamese lung adenocarcinomas, *RET* abnormalities have been identified in 2.8%, which is a light higher than previous publications. There is no significant difference in *RET* rearrangement among cohorts subdivided by patient characteristics, including age, gender, tumor site and smoking status. A larger study is needed to validate our results.

c-Met or MET, also known as hepatocyte growth factor receptor, is a cell transmembrane receptor. Activation of MET can induce cell proliferation, survival, migration, invasion, angiogenesis, and transition from epithelial to mesenchymal through RAS/ERK/MAPK, PI3K/AKT, Wnt/β-catenin and STAT pathways [22]. *MET* skipping exon 14 occurs 2-3% in NSCLC, which may prolong the activation of MET signal to downstream pathways. [18]. Smokers and older patients more commonly harbor this mutation. In our research, the frequency of *MET* skipping exon 14 was identified in 2.2% of adenocarcinoma lung cancer patients. Alterations of *MET* were slightly more common in patients older than 60 years, late stages, and cigarette-addicted patients but there is not significant statistics.

EGFR mutation in lung cancer widely applied in Vietnam as well as other countries, but alterations of *ALK, ROS1, RET* and *MET* detections are new research areas for NSCLC in Vietnam. Our study was one of pioneer research analyzing oncogenic driver mutations of five genes in Vietnamese lung adenocarcinomas. The prevalence abnormalities of oncogenic genes were identified in the most common subtype of lung cancer in Vietnamese patients. The frequencies of genetic alterations seem similar to the previous publications for Asian Ethics. Also, there are differences in mutation rates among cohorts that different in clinicopathological characteristics. However, the number of patients in the research and targeted genes should be expanded to get valid results.

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AUTHOR CONTRIBUTIONS

LDV and QNN: Conception and Design of the experiments. LDV: Methodology and Data analysis, LDV: Data curation and Writing – original draft, QNN: Writing – review and editing. QNN: Supervision. All authors reviewed final version of the manuscript.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

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