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Original contribution

Detection of *RAS* and *RAS*-associated alterations in primary lung adenocarcinomas. A correlation between molecular findings and tumor characteristics☆,☆☆

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Summary *Rat sarcoma* (*RAS*) and *RAS*-associated pathways play important roles in the pathogenesis of lung cancers and in the development of targeted therapies. However, the clinical significance of *RAS* pathways is still not fully understood. We investigated the *RAS*-associated molecular aberrations in primary lung adenocarcinomas and correlated molecular findings with clinicopathological characteristics of tumors. A total of 220 surgically resected tumors were identified for which a lung cancer molecular panel (testing 7 genes by next-generation sequencing and 3 genes for rearrangement by fluorescence in situ hybridization) had been performed. The overall molecular alterations were detected in 143 cases (65.00%), including 58 cases (26.36%) of *KRAS*, 40 cases (18.18%) of *EGFR*, 24 cases (10.91%) of *BRAF*, 8 cases (3.64%) of *PIK3CA*, 7 cases (3.18%) of *NRAS*, 6 cases (2.73%) of *ALK* alterations. *KRAS*, *BRAF*, *NRAS*, and *PIK3CA* mutations were more commonly seen in smokers and occurred with much higher rates than previously published data. $BRAF^{V600E}$ mutations were commonly seen in female smokers, whereas, $BRAF^{non\text{-}V600E}$ mutations were seen in both male and female smokers with moderately to poorly differentiated tumors. *PIK3CA* mutations were predominantly occurred in p.E545K and p.E542K on exon 9 in moderately to poorly differentiated tumors.

1. Introduction

The Cancer Genome Atlas (TCGA) studies have demonstrated that many driver oncogenes are involved in non–small cell lung carcinoma (NSCLC), particularly in lung adenocarcinoma

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(ADC), such as *epidermal growth factor receptor* (*EGFR*), *anaplastic lymphoma kinase* (*ALK*), *phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha* (*PIK3CA*), *Kirsten rat Sarcoma viral oncogene* (*KRAS*), *neuroblastoma RAS viral (v-ras) oncogene homolog* (*NRAS*), *V-raf murine sarcoma viral oncogene homolog B* (*BRAF*), *mesenchymal-epithelial transition factor* (*MET*), *rearranged during transfection* (*RET*), *ROS proto-oncogene 1* (*ROS1*), *Ak strain transforming 1 or AKR mouse thymoma kinase* (*AKT1*), *human epidermal receptor growth factor 2* (*HER2* or *ERBB2*), and others [1-7]. Based on these studies, the number of US Food and Drug Administration–approved targeted therapies for NSCLCs has increased rapidly, particularly in targeting tumors with aberrations in *EGFR* and *ALK* signaling pathways [6-9]. More recently, immuno check-point inhibitor therapies have also been increasingly used in patients with advanced lung cancer [10]. However, lung cancer is still the leading cause of cancer-related deaths in the United States and worldwide [11]. Therefore, understanding the molecular alterations and susceptibility to targeted treatment in NSCLC is a critical step to improve patient survival.

In addition to *EGFR* and *ALK* signaling pathways, *RAS and RAS*-associated pathways play a critical role in the tumorigenesis in a variety of cancers [6,7]. For example, aberrant RAS pathways have been detected in 50% to 90% of pancreatic cancers, 36% of colorectal cancers, 14% of thyroid cancers, 19% of NSCLCs, 7% of breast cancers, and 15% of prostate cancers [6,7]. RAS proteins are a family of guanosine triphosphate (GTP) hydrolase, which are activated by cell surface receptors via the interaction of guanosine diphosphate and GTP [7]. The GTP-RAS complex is then able to recruit and activate several downstream intracellular signaling pathways, such as the RAS-RAF-MEK-ERK pathway (also known as the mitogen-activated protein/extracellular signal-regulated kinase [MAPK/ERK] pathway) and phosphoinositide-3 kinase (PI3K) pathway [6-9,12]. In the RAS-RAF-MEK-ERK pathway, the RAS regulates the activity of RAF proteins and several nuclear transcription factors and is involved in cell proliferation, apoptosis, invasion, and metastasis [6,9,12]. In the PI3K pathway, the catalytic p110 subunit of the PI3K regulates the transition of phosphorylates phosphatidylinositol 4,5-bisphosphate to phosphatidylinositol (3,4,5)-triphosphate, leading to the activation of the PI3K/AKT/mammalian target of rapamycin pathway, which promotes aberrant cellular proliferation, apoptosis, and migration [7,12]. Taken together, molecular alterations of RAS-associated signaling pathways play complex roles in the pathogenesis of cancers (Fig. 1).

Very recently, based on the genetic discoveries and current advances in lung cancer research, the College of American Pathologists/the International Association for the Study of Lung Cancer/the Association for Molecular Pathology have reevaluated and updated the evidence-based guidelines for lung cancer [13]. The guideline emphasizes the testing of aberrant *RAS* and *RAS*-associated mutations in NSCLC for the targeted therapy. It recommends that *BRAF* testing should be performed on advanced ADCs, irrespective of clinical characteristics, and *KRAS* testing should be included as part

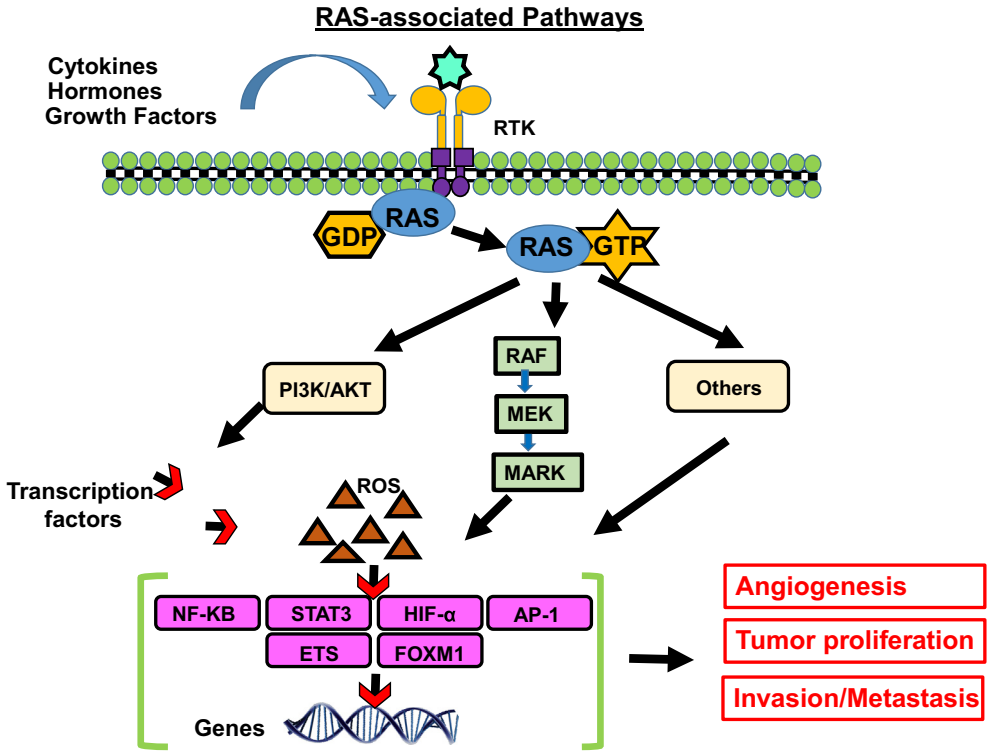


Fig. 1 Intracellular signaling pathways in RAS-associated molecules. Both the RAS-RAF-MEK-ERK and PI3K pathways are highlighted.

of larger testing panels performed either initially or when routine *EGFR*, *ALK*, *BRAF*, and *ROS1* testing is negative [13].

Although many studies have been published to address clinical role of *RAS* and *RAS*-associated signaling pathways in lung cancer [14-17], the overall role of *RAS* and RAS-associated aberrant proteins is still not well characterized. We investigated *RAS*-associated alterations in the primary lung ADCs and correlate molecular findings with patients' clinical characteristics and tumor differentiations.

2. Materials and methods

2.1. Case collection

A retrospective computer search of the pathology archives was performed for the identification of surgical resected primary lung ADCs for which a lung molecular panel (including testing aberrant of 7 genes by next-generation sequencing [NGS] and rearrangement of 3 genes by fluorescence in situ hybridization [FISH]) was performed over a 24-month period. The World Health Organization and International Association for the Study of Lung Cancer/American Thoracic Society classification criteria were used for determination of histologic subtypes and pathological stages of lung NSCLC [18]. Immunohistochemical markers, including TTF1, Napsin A, and P40, were also used to aid in the morphologic subclassifications of ADCs. Based on the predominant growth pattern and nuclear features, we also separated tumors into well-differentiated, moderately differentiated, and poorly differentiated groups. For example, in tumors of acinar subtype, a well-differentiated tumor is considered to have more than 70% glandular formation and mild to moderate nuclear atypia, a moderately differentiated tumor is considered to have 20% to 70% glandular formation and moderate nuclear atypia, and a poorly differentiated tumor is considered to have less than 20% glandular formation and markedly nuclear atypia. Other subtypes of ADC were also objectively divided into 3 groups according to the tumor differentiation and nuclear features. The study was approved by the institutional review board of the Johns Hopkins Medical Institutions.

2.2. Tumor tissue preparation

Surgically resected tumor tissues were fixed in 10% neutral-buffered formalin, embedded with paraffin, and processed in the histology laboratory. All tumor tissue blocks were cut at 5-μm thickness and stained with hematoxylin and eosin for morphologic diagnosis. Unstained slides were cut at 5-μm thickness and used for the molecular analysis in our molecular laboratory.

2.3. Molecular Analysis

The NGS is routinely performed on lung cancer patients at our institution when the sample is adequate and the testing is clinically indicated. The cellularity on the hematoxylin and eosin–stained slides is evaluated by the American Board of Pathology–certified pathologists. In general, if neoplastic cells are more than 20% of all nucleated cells, then the specimen is considered suitable for testing.

DNA from tumor cells was harvested from 5 unstained slides. NGS was conducted using the AmpliSeq Cancer Hotspot Panel (Life Technologies) (v2) for targeted multigene amplification, as described previously [19]. Briefly, we used the Ion AmpliSeq Library Kit 2.0 for library preparation, Ion OneTouch 200 Template Kit v2 DL, and Ion OneTouch Instrument for emulsion polymerase chain reaction and template preparation, and the Ion Personal Genome Machine 200 Sequencing Kit with the Ion 318 Chip and Personal Genome Machine as the sequencing platform (Life Technologies, Carlsbad, CA). The DNA input was up to 30 ng, as measured by Qubit 20 Fluorometer (Life Technologies).

Sequencing data were analyzed using Torrent Variant Caller (Life Technologies) and direct visual inspection of the binary sequence alignment/map file using the Broad Institute's Integrative Genomics viewer (Cambridge, MA, USA). For single-nucleotide variants, the limit of detection was at least 5%; for insertions and deletions, the limit of detection was at least 10%. Rare variants without COSMIC references were confirmed by either Sanger sequencing or Pyrosequencing analyses. Lung cancer specimens were tested for *AKT*, *BRAF*, *EGFR*, *ERBB2*, *KRAS*, *NRAS*, and *PIK3CA* genes (lung cancer panel).

Rearrangements of *ALK*, *ROS1*, and *MET* were also tested by FISH analysis, if EGFR and KRAS testing showed negative results. *ALK* FISH was performed using a Vysis ALK Break Apart FISH probe (Abbott Molecular, Des Plaines, IL) following the manufacturer's instructions. Briefly, a total of 50 tumor nuclei were analyzed after the staining of FISH dual-color probe for a locus on 2p23. The separation of the 5′*ALK* and 3′*ALK* signals in tumor cells was scored as negative (<10% of cells) and positive (>50%), respectively [20]. If results fell in the range of 10% to 50%, an additional 50 tumor cells were scored. If a total of more than 15% of tumor cells with separation were found, then the case was considered as positive. ROS1 and MET rearrangements assay were performed similarly but using different probes.

3. Results

3.1. Clinical information

A total of 220 surgical resected primary lung ADCs were included. Patients' clinical information is summarized in Table 1. The median age of patients was 67.5 years, ranging from 33 to 86 years. The male-to-female ratio was 1:1.53 (87 male and 133 female patients). In our study, 129 patients were smokers or ex-smokers, and 85 patients were nonsmokers, whereas 6 patients had an unspecified history of smoking. Most tumors were located in the peripheral areas of the lung (147/220 tumors).

Among tumors, 103 cases were pT1, 82 cases were pT2, 25 cases were pT3, and 10 case were pT4 tumors. The median

Table 1 Demographic information of patients and pathological stage of tumors

Characteristics	No. of cases (%)
Age (y), median (range): 67.5 (33-86)	N/A
Sex	
Male	87 (39.55)
Female	133 (60.45)
Smoking history	
Smoker	129 (58.64)
Nonsmoker	85 (38.63)
Unspecified	6 (2.73)
Location of tumors	
Central	69 (31.36)
Peripheral	147 (66.82)
Unspecified	4 (1.82)
Tumor differentiation	
Well differentiated	30 (13.64)
Moderately differentiated	88 (40.00)
Poorly differentiated	65 (29.55)
Unspecified	37 (16.82)
Pathological stage of tumors	
pT1	103 (46.82)
pT2	82 (37.27)
pT3	25 (11.36)
pT4	10 (4.55)
Total	220 (100)

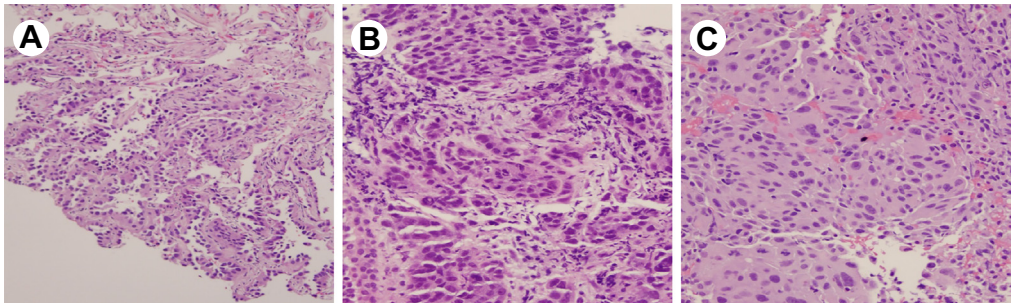
Abbreviation: N/A, not applicable.

size of tumors was 3.28 cm, ranging from 0.5 to 9.0 cm. The subtypes of ADCs were as follows: acinar (75 cases), mixed (68 cases), nonmucinous ADC with lepidic pattern mucinous (17 cases), solid (23 cases), and others (37 cases). Of the tumors, 65 cases were poorly differentiated, 88 cases were moderately differentiated, 30 cases were well-differentiated tumors, and 37 cases were unspecified. Representation of tumor morphology is shown in Fig. 2.

3.2. Molecular findings

In our lung molecular panel, alterations of *AKT*, *BRAF*, *EGFR*, *ERBB2*, *KRAS*, *NRAS*, and *PIK3CA* were tested by NGS, and rearrangements of *ALK*, *ROS1*, and *MET* were tested by FISH analysis. A total of 143 tumors (65.00%) were found to harbor alterations of *BRAF*, *EGFR*, *KRAS*, *NRAS*, and *PIK3CA*, or *ALK* rearrangements. Of these cases, 95 patients (66.43%) were female and 48 patients (33.57%) were male. Ninety-four patients (65.73%) were smokers, and 49 patients (34.27%) were nonsmokers (Table 2). Tumors with genetic alterations included 20 cases (13.99%) of well-differentiated, 56 cases (39.16%) of moderately differentiated, 50 cases (34.97%) of poorly differentiated, and 17 cases (11.89%) of unspecified tumors (Fig. 2).

In the RAS pathways, *KRAS* mutations were detected in 58 cases (26.36%), including alterations in codons 12, 13, and 61 (Table 3). The *G12X* alteration was observed in 86.21% (50/58 cases) of *KRAS* mutations, and the *G13D* alteration was observed in 8.62% (5/58 cases) of *KRAS* mutations. *Q61H* and *Q61L* mutations were also detected in 2 cases and 1 case, respectively. *KRAS* mutations were more commonly seen in smokers (Table 2) and in moderately (27 cases) to poorly (20 cases) differentiated tumors, with only 4 cases being well-differentiated tumors. *BRAF* mutations were detected in 24 cases (10.91%), including 21 smokers and 3 nonsmokers (Table 2). Of *BRAF* alterations, *V600E* mutations were detected in 9 cases of well- to moderately differentiated tumors,



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Genes	Well-diff Cases (%)	Moderately-diff Cases (%)	Poorly-diff Cases (%)	Unspecified Cases (%)	Total Cases (%)
EGFR	10 (25.00 %)	13 (32.50 %)	9 (22.50 %)	8 (20.00 %)	40 (100%)
KRAS	4 (6.90 %)	27 (46.55 %)	20 (3.48 %)	7 (12.07 %)	58 (100%)
BRAF	4 (16.67 %)	12 (50.00 %)	7 (29.17 %)	1 (4.17%)	24 (100%)
NRAS	1 (14.29 %)	1 (14.29 %)	5 (71.43 %)	0	7 (100%)
PIK3CA	1 (12.50 %)	2 (25.00 %)	5 (62.50 %)	0	8 (100%)
ALK	0	1 (16.67 %)	4 (66.67%)	1 (16.67%)	6 (100%)
Total	20	56	50	17	143

Fig. 2 Summary of mutations and tumor differentiations. A, Representative morphology of well-differentiated ADC with a predominantly acinar and lepidic growth pattern. B, A moderately differentiated ADC with focal glandular growth pattern. C, A poorly differentiated ADC with a predominantly solid growth pattern and markedly nuclear atypia. D, Summary of molecular findings and tumor differentiation.

Table 2 Correlation of molecular findings between smokers and nonsmokers

Genes	Smoker (n = 94)		Nonsmoker (n = 49)	
	Male, no. of cases (%)	Female, no. of cases (%)	Male, no. of cases (%)	Female, no. of cases (%)
BRAF (n = 24)	5 (20.83)	16 (66.67)	0	3 (12.50)
NRAS (n = 7)	2 (28.57)	5 (71.43)	0	0
KRAS (n = 58)	20 (34.48)	28 (48.28)	3 (5.17)	7 (12.07)
EGFR (n = 40)	6 (15.00)	3 (7.50)	6 (15.00)	25 (62.50)
PIK3CA (n = 8)	5 (62.50)	2 (25.00)	0	1 (12.50)
ALK (n = 6)	0	2 (33.33)	1 (16.67)	3 (50.00)
Total (n = 143)	38	56	10	39

Table 3 Detection of *KRAS* mutations in primary lung ADC

KRAS mutations	Nucleotide change	Codon change	Amino acid change	No. of cases (%)
Codon 12	GGT → TGT	G12C	Glycine to cysteine	18 (31.03)
	GGT → GCT	G12A	Glycine to alanine	14 (24.14)
	GGT → GTT	G12V	Glycine to valine	10 (17.24)
	GGT → GAT	G12D	Glycine to aspartic acid	5 (8.62)
	GGT → TTT/C	G12F	Glycine to phenylalanine	2 (3.45)
	GGT → AGT	G12S	Glycine to serine	1 (1.72)
Codon 13	GGC → GAC	G13D	Glycine to aspartic acid	5 (8.63)
Others	CAA → CAC/T	Q61H	Glutamine to histidine	2 (3.45)
	CAA → CTA	Q61L	Glutamine to leucine	1 (1.72)

with 6 cases being smokers, whereas non-*V600E* mutations were detected in 15 cases of moderately to poorly differentiated tumors, with all cases being smokers (Fig. 3). Interestingly, non-*V600E* mutations were more commonly seen in smokers. *NRAS* mutation was detected in 7 tumors (3.18%), 6 of them were moderately to poorly differentiated tumors and 1 was well-differentiated tumor (Fig. 2). Interestingly, all *NRAS* mutations were detected in smokers (Table 2).

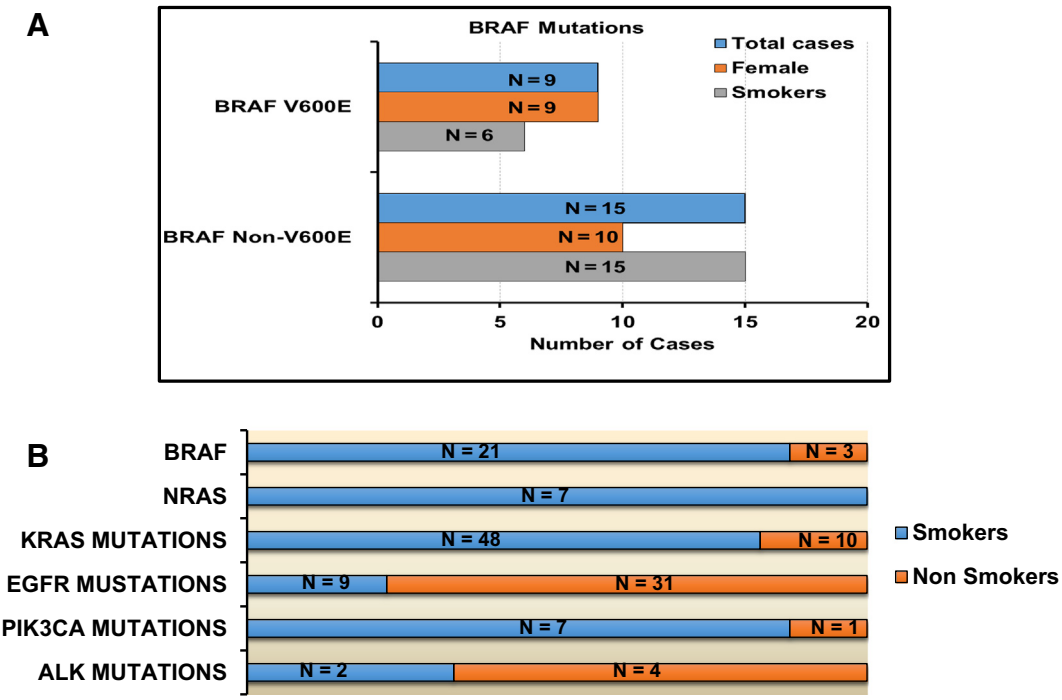

Fig. 3 Correlation of molecular findings with clinical information. A, Characteristics of *BRAF* mutations in smokers and nonsmokers. B, Correlation of molecular findings with patients' smoking status.

In the PI3K pathway, *PIK3CA* mutations were detected in 8 cases (3.64%), 7 of these mutations were from patients with a history of smoking (Table 2). Most of these tumors were moderately to poorly differentiated tumors, including 2 and 5 cases, respectively. Of *PIK3CA* mutations, 3 cases were p.E545K and 2 cases were p.E542K on the exon 9, and 2 cases were p.H1047R and 1 was p.H1047L on the exon 20.

No alterations were detected in *AKT* and *ERBB2* genes. *EGFR* alterations were detected in 40 cases (18.18%) and mostly observed in nonsmokers (25 female and 6 male patients; Table 2). These tumors revealed varied degrees of differentiation, including 10 cases of well-differentiated, 13 cases of moderately differentiate, 9 cases of poorly differentiated, and 9 cases of unspecified tumors (Fig. 2). Furthermore, the *ALK* rearrangement was detected in 6 cases (2.73%). Four cases were poorly differentiated, 1 was moderately differentiated, and 1 was an unspecified tumor type (Fig. 2, Table 2). Four cases were smokers, and the other 2 cases were nonsmokers.

4. Discussion

RAS genes are a family of proto-oncogenes and are frequently mutated in human cancers including lung cancers [6-9,17]. RAS proteins function as molecular switches in many intracellular signaling pathways responsible for cell proliferation and survival. Among *RAS* genes, *KRAS* is the most frequently mutated genes in most cancers such as colon and pancreatic cancer where 50% to 90% of tumors harbor mutations [6-9]. *NRAS* mutations are more commonly found in hematopoietic tumors. *BRAF* mutations are found in thyroid, skin, breast, and ovarian cancers, and *PIK3CA* alterations are found in breast, urinary tract, endometrial, and penile cancers [6-9]. The TCGA data and other studies have demonstrated that genetic alterations were detected in 62% of primary lung ADCs, including *KRAS* (32.2%), *EGFR* (11.3%), *BRAF* (7.0%), *ALK* (1.3%), *NRAS* (0.4%), and *PIK3CA* (1%-2%) [1,9,17].

Our previous study has demonstrated that *KRAS* mutations were detected in 28.3% to 40% in primary and metastatic lung ADCs [14,15]. In this study, *KRAS* mutations were detected in 58 cases (26.36%), involving codons 12, 13, and 61, and mostly by substituting an amino acid on these codons. The *G12X* mutations were the most common findings, involving 50 cases (86.21%). The *G13D* mutations were detected in 5 cases (8.62%), and *Q61X* mutations were detected in 3 cases (5.17%), which was higher than the previously reported rate of less than 1% [1,2,6,7,17]. It has been reported that the *G12C* mutation is commonly seen in lung cancer patients with a strong history of smoking [6,7]. Similarly, we also found a strong association of this mutation among patients with history of smoking. It was also reported that in advanced colon cancer, *G13D* mutation has prognostic benefit for anti-EGF receptor cetuximab-based therapy [6]. Although it has not been reported in lung cancers, the finding indicates the clinical importance of discriminating different types of *KRAS* mutations in lung cancers [21]. In our study, the prevalence of *KRAS* mutations was more commonly observed in moderately to poorly differentiated tumors. However, our study has a limited number of cases, and a large-scale study is necessary to validate our findings. Finally, the clinical significance of *KRAS* mutations is still controversial. For example, some clinical trials have reported that *KRAS* mutations are a negative predictive marker for insensitivity to EGFR tyrosine kinase inhibitor (TKI) therapy and associated with poor prognosis, whereas other studies have demonstrated that *KRAS* mutations are neither prognostic nor predictive for the benefit of the EGFR TKI therapy [21,22]. Although several agents are being tested in clinical trials for *KRAS*-mutated lung cancers, there are still no direct anti-*KRAS* therapies available.

In our study, *BRAF* mutations were more common in female patients (19/24 cases) and smokers (21/24 cases). The V600E mutations (valine to glutamic acid substitution at residue 600) were seen in female patients (9/9 cases) and smokers (6/9 cases). Our findings are similar to the previous studies that the mutation has commonly involved in female patients [23-26]. The non-V600E BRAF mutant was detected in 15 cases, and all of them were associated with smoking history. In addition to smoking history, we also found that V600E mutations were more commonly seen in well-differentiated tumors, whereas non-V600E mutations occurred more often in moderately to poorly differentiated tumors. In previous studies, it is also reported that tumors with V600E mutations showed predominantly micropapillary features and associated with poor prognosis [24,27,28]. However, we did not find these features in our study. A consensus has not been reached on the morphologic characteristics of tumor harboring *BRAF* mutations; however, large cohort studies are needed to find any association between *BRAF* mutations and lung cancer prognosis. Of note, the Food and Drug Administration has recently approved a regimen of combination of dabrafenib and trametinib for targeting tumor with $BRAF^{V600E}$ mutations in NSCLC [29].

We detected 7 cases (3.18%) of *NRAS* mutations. All of them were found in smokers, including 5 women and 2 men. Our observation of the mutational rate was higher than the previously observed rate of than less 1% in NSCLC [1,7-9]. Its clinical significance is still unclear, and currently, no effective targeted therapies have been identified.

In primary NSCLC, recent data have shown that alterations in the PI3K pathway are much more frequently detected in squamous cell carcinoma than in ADC [1,2,8,9,12]. The TCGA data have shown that 16% of squamous cell carcinoma had PIK3CA mutations, with exon 9 being the most frequently mutated location [1,2]. PIK3CA mutations are much rarer in ADCs and only found in 1% to 2% tumors. Our previous study of metastatic NSCLC has found that the PIK3CA mutations were detected in 6.1% of tumors [30]. In current study of primary lung ADC, *PI3KCA* mutations were detected in 8 cases (3.64%), including 7 smokers and 1 nonsmokers, and 7 cases were moderately to poorly differentiated tumors. The loci and affected domains of PIK3CA alterations include 3 cases of p.E545K and 2 cases of p.E542K on the exon 9, and 2 cases

of p.H1047R and 1 case of p.H1047L on the exon 20. Our findings are consistent with the reports that the most frequent mutations are p.E545K and p.E542K on exon 9 [1,2,8,9,12]. It has been reported that *PIK3CA* mutations may interact with *EGFR* and *KRAS* mutations [12,30-32]. The interactions between these mutations are complex but therapeutically relevant because PIK3CA mutations have been detected in approximately 5% of patients with an acquired resistance to TKI therapy [31,32]. It is also reported that the *PIK3CA* mutation p.E545K on exon 9 is associated with resistance to TKI therapy in patients with concurrent EGFR-sensitive mutations, whereas NSCLCs with *PIK3CA* mutations that are *EGFR/KRAS* wild-type are associated with significantly worse survival [33].

Finally, *EGFR* alterations were detected in 40 cases (18.18%), including 9 cases of smokers and 31 cases of nonsmokers. Our findings are consistent with previous studies that EGFR mutations are more commonly seen in female patients and nonsmokers or in patients with very limited exposure to smoking. *ALK* rearrangements were detected in 6 cases (2.73%), and our detection rate was slightly higher than the previously reported rate of 1.3% in TCGA study [1]. The mutation was more commonly observed in women (5/6 cases). The median age of patients was 67 years (range of 51-81 years). Our findings are different from previous studies, which reported that the mutation was more commonly seen in younger patients (median age of 50 years) and in nonsmokers [34]. In the TCGA study, *ROS1* alterations were previously reported in 1% to 2% of cases [1]. Although we did not detect *ROS1* aberrations in our study, it is still necessary to include *ROS1* in the lung molecular panel owing to the clinical benefit for patients, as the tumor harboring has been reported to be highly sensitive to ALK TKI, that is, crizotinib therapy due to a high degree of homology between the ALK and ROS tyrosine kinase domains [35].

In summary, a total of 143 mutations (65%) were detected in 220 primary lung ADCs. The mutational rate of lung ADC in our study is similar to the TCGA data. The prevalence of molecular aberrant identified in our study includes 58 cases (26.36%) of *KRAS*, 40 cases (18.18%) of *EGFR,* 24 cases (10.91%) of *BRAF*, 8 cases (3.64%) of *PIK3CA*, 7 cases (3.18%) of *NRAS*, and 6 cases (2.73%) of *ALK* alterations. *BRAF* and *NRAS* mutations were more commonly seen in smokers and occurred in much higher rate than previously published data. $BRAF^{V600E}$ mutations were commonly seen in well-differentiated tumors, whereas $BRAF^{non\text{-}V600E}$ mutations were more commonly seen in moderately to poorly differentiated tumors. *PI3KCA* mutations were detected predominantly in smokers, and the most frequent mutations are p.E545K and p.E542K on exon 9. *ALK* rearrangements in our study were found in patients older than the previously reported age of 50 years. Clinical significances of these mutations are still not fully discovered, and further investigations are critical to understand these mutations and their association with tumor morphologic characteristics and patient demographic features.

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