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Detection of *EGFR* and *KRAS* Mutation by Pyrosequencing Analysis in Cytologic Samples of Non-Small Cell Lung Cancer

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EGFR and *KRAS* mutations are two of the most common mutations that are present in lung cancer. Screening and detecting these mutations are of issue these days, and many different methods and tissue samples are currently used to effectively detect these two mutations. In this study, we aimed to evaluate the testing for *EGFR* and *KRAS* mutations by pyrosequencing method, and compared the yield of cytology versus histology specimens in a consecutive series of patients with lung cancer. We retrospectively reviewed *EGFR* and *KRAS* mutation results of 399 (patients with *EGFR* mutation test) and 323 patients (patients with *KRAS* mutation test) diagnosed with lung cancer in Konkuk University Medical Center from 2008 to 2014. Among them, 60 patients had received both *EGFR* and *KRAS* mutation studies. We compared the detection rate of *EGFR* and *KRAS* tests in cytology, biopsy, and resection specimens. *EGFR* and *KRAS* mutations were detected in 29.8% and 8.7% of total patients, and the positive mutation results of *EGFR* and *KRAS* were mutually exclusive. The detection rate of *EGFR* mutation in cytology was higher than non-cytology (biopsy or resection) materials (cytology: 48.5%, non-cytology: 26.1%), and the detection rate of *KRAS* mutation in cytology specimens was comparable to non-cytology specimens (cytology: 8.3%, non-cytology: 8.7%). We suggest that cytology specimens are good alternatives that can readily substitute tissue samples for testing both *EGFR* and *KRAS* mutations. Moreover, pyrosequencing method is highly sensitive in detecting *EGFR* and *KRAS* mutations in lung cancer patients.

Keywords: *EGFR*; *KRAS*; Pyrosequencing; Cytology Specimen; Mutation Test; Lung Cancer

INTRODUCTION

Lung cancer is one of the most common cause of cancer-related death worldwide (1). Its median survival for non-small cell lung cancer (NSCLC) is estimated to be 8 to 10 months, and 1-year survival to be only 30% to 35% with standard chemotherapeutic treatment (2-5). Some of the NSCLCs have specific mutated oncogene which is considered to be the main genetic defect that leads to cancer (6). The two most commonly mutated oncogenes in lung cancer are epidermal growth factor receptor (*EGFR*) and Kirsten rat sarcoma viral oncogene homologue (*KRAS*) (6). EGFR has a pivotal role as a receptor tyrosine kinase, controls signal pathways that controls cell growth and proliferation. As a consequence, mutations in the kinase domain of *EGFR* gene (exons 18-21) strongly correlate with improved overall survival and disease-free survival in patients with NSCLC who receive the EGFR tyrosine kinase inhibitors erlotinib or gefitinib as treatment therapy (7,8). These mutations are commonly associated with never or non-smoker, adenocarcinomatous morphology, and Asian ethnicity (6,8). On the other hand, unlike those of *EGFR* mutant, *KRAS* mutations are usually found in those with significant smoking history (6,9). Moreover, *KRAS* mutations, which encodes a GTPase downstream of EGFR, are associated with primary resistance to tyrosine kinase inhibitors in patients with NSCLC, which appears to be mutually exclusive to *EGFR* mutations in NSCLC (6,9). Taken together, current evidence suggests that EGFR and *KRAS* mutations define distinct subgroups of NSCLC patients, with different responses to EGFR- targeted therapies.

In these backgrounds, testing for *EGFR* and *KRAS* mutations have now become a routine practice for therapeutic management (10). Sensitive, rapid, and at the same time, reliable methods for detecting these mutations are required for targeted treatment. Thus now, the most frequently used conventional method for detecting *EGFR* and *KRAS* mutations is considered to be direct DNA sequencing method (11). However, this technique has some limitation, including frequent interference of nonmalignant cells, and is not necessarily practical for clinical use with suboptimal sensitivity (11,12). Pyrosequencing is a simple and accurate DNA sequencing technique based on detection of released pyrophosphate during DNA synthesis (13).

In the past, cytologic specimens have not been widely used

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for mutational sequence analysis due to sparse cellularity (12). But in recent years, cytology specimens are being more frequently used for mutational tests, especially when cytological materials are the only available tissues for molecular testing (14). Several studies reported that cytology specimens also yield comparative results similar to surgical specimens (5,10,14). Overall, preservation and quality of the DNA extracted seemed to matter more than the actual number of tumor cells present in the samples. In a recent consensus for mutation testing in NSCLC, there was agreement that the quality of amplifiable DNA is more important than its quantity.

In this study, we aimed to evaluate the testing for *EGFR* and *KRAS* mutations by pyrosequencing method, and compared the yield of cytology versus histology specimens in a consecutive series of patients with NSCLC in Konkuk University Medical Center, Seoul, Korea.

MATERIALS AND METHODS

Patient selection

This retrospective study examined 446 patients who were diagnosed as lung cancer in Konkuk University Medical Center, Seoul, Korea from January, 2008 to September, 2014. The eligible criteria were as follows: a) patient who presented with lung mass and diagnosed as primary or metastatic cancer with the methods of cytology, biopsy, and excision, b) cases which *EGFR* and *K-RAS* mutation studies were done. We also included the specimens, such as lymph nodes, from the metastatic sites with primary lung cancer. According to the above criteria, total number of 399 and 323 patients who had *EGFR* and *KRAS* mutation tests were included in the study, respectively. Among them, 60 patients had received both *EGFR* and *KRAS* mutation studies.

Clinicopathological analysis

To evaluate the clinicopathologic features of the patients, medical records of 399 (patients who had *EGFR* mutation test) and 323 (patients who had *KRAS* mutation test) patients were reviewed including patient age, gender, history of smoking, pathologic diagnosis, and most importantly, the diagnostic methods.

The smoking history was determined according to pack years (py), and subdivided into current, ex-smoker, non-smoker. We defined current smoker as more than 10 py history, and ex-smoker who has smoked greater than 100 cigarettes in their lifetime, does not currently smoke, but used to smoke daily. Never smoker are defined as who have never smoked a cigarette or who smoked fewer than 100 cigarettes in their entire lifetime.

The subtypes of diagnostic methods are divided into three categories: Biopsy, Cytology, and Excision. In 'Biopsy' category, the specimens includes small biopsy specimen from bronchoscopic biopsy, transbronchial lung biopsy, percutaneous needle biopsy, pleural biopsy or needle biopsy from metastatic sites. The 'Cytology' includes the cytologic specimens from sputum, bronchial washing/brushing, pleural fluids, aspiration biopsy cytology of primary or metastatic sites. 'Excision' category includes specimens form excisional surgical biopsy such as segmentectomy, lobectomy, pneumonectomy and metastatectomy.

The pathologic diagnoses were made by individual pathologists. Diagnoses from biopsy specimens were reviewed by experienced lung pathologist, and classified according to the WHO classification (WSK). Adenocarcinoma subtypes were evaluated separately from non-adenocarcinomatous lesions, for it is well known that both *EGFR* and *KRAS* mutations have tendency to occur primarily in adenocarcinomatous subtype.

EGFR and *KRAS* mutation analysis – pyrosequencing method

In all the cytologic and histologic samples, target tumor rich areas were marked by microscopic examination of pathologists. The tumor cells were scraped from the archived slides after the coverglass and xylene were removed. Microdissections on both cytological and tissue slides were done with 26-guage needle. The DNA was extracted from the tumor cells by the following in order – 1) Transfer the collected cells to 30 μL DNA isolation buffer, 2) Add 0.3 μL (20 mg/mL) Proteinase K and vortex, 3) Incubate at 56°C until complete lysis is done, 4) Incubate at 100°C for 20 minutes, 5) Immediately centrifuge at 12,000 rpm for 10 minutes at 4°C, 6) Transfer supernatant (DNA) to a new tube. *EGFR* and *K-RAS* mutation were analyzed by pyrosequencing method.

Statistical analysis

Statistical analysis was performed using SPSS version 18.0 for Windows (SPSS Inc., Chicago, IL, USA). Chi-square test and Fisher's exact test were used to determine correlations between *EGFR* and *K-RAS* mutation status and clinicopathological parameters. A P value of < 0.05 was considered statistically significant.

Ethics statement

The study approval was obtained from the institutional review board of Konkuk University Medical Center (KUH1210020). The informed consent was exempted by the board.

RESULTS

EGFR mutations and clinicopathologic characteristics

Of the 399 patients who had EGFR mutation test, 254 were male, and 105 were female (male:female = 254 [63.7%]:105 [36.3%]). The patient age ranged from 25 to 90 years old, with median age of 65.47 years. Among the three diagnostic tools, majority of cases (269, 67.4%) were biopsy specimen, followed by cytology specimens; 66 (16.5%), and resection specimens; 64 cases (16.1%).

Of them, 119 patients were positive for *EGFR* mutation (29.8%), and among them, 72 patients were female (60.5%, n = 72). Deletion in exon 19 (E746_A750) was the most frequently found mutation (48 cases, 40.3%), followed by L858R point mutations in exon 21 in 35 cases (29.4%). The detailed *EGFR* mutation profiles are summarized in Supplementary Table 1.

The *EGFR* mutation and smoking history showed strong inverse correlation ($P < 0.001$); 65% was non-smoker, and only 35% had history of smoking. The detection rates of *EGFR* mutation were highest in cytologic samples; 32 out of 66 samples (48.5%), resection specimens the 2nd highest; 21 of 43 (32.8%), and then biopsy specimens; 66 out of 269 (32.5%). As a result, cytology was more sensitive than non-cytology (histology) samples in detecting *EGFR* mutations (cytology: 48.5%, non-cytology: 26.1%). Table 1 summarizes the detailed information about patients who had *EGFR* mutation test.

Of the various histologic subtypes in 399 cases, adenocarcinoma was the most common subtype, being more than 70% of the whole samples (70.4%, 281 out of 399), followed by squamous cell carcinoma (n = 69), large cell carcinoma (n = 15), and small cell carcinoma (n = 12). In 281 adenocarcinoma cases, 111 cases were *EGFR* mutation positive (39.5%). Five of 69 squamous cell carcinoma were *EGFR* positive, and none of large cell and small cell carcinoma were *EGFR* positive. Among 119 *EGFR* mutation positive cases, 111 cases were diagnosed as adenocarcinoma. Five cases were squamous cell carcinoma, followed by 2 adenosquamous cell carcinoma, and 1 non-small cell carcinoma. The details about the *EGFR* mutation and the pathologic subtypes are shown in Table 2.

On the basis of the fact that *EGFR* mutations most frequently occur in adenocarcinoma, we compared the diagnostic yield of detecting adenocarcinoma in cytology, biopsy, and resection

Table 1-1. Patients demographics with *EGFR* mutation (n = 399 for *EGFR*)

Demographic parameters	EGFR Mu n = 119 (29.82%)	EGFR WT n = 280 (70.18%)	Total n = 399	*P* value
Age, yr (range)	65.23 (34-90)	62.93 (25-88)	64.71 (25-90)	0.587
Sex (M:F)				0.064
Male	47 (39.50)	207 (73.92)	254 (63.65)	
Female	72 (60.50)	73 (26.08)	145 (36.35)	
Smoking history				< 0.001
Current	16	68	84	
Ex-smoker	21	46	67	
Non-smoker	70	152	222	
Unknown	12	14	26	
Smoker	37 (34.57)	114 (42.85)	151 (40.48)	
Non-smoker	70 (65.43)	152 (57.15)	222 (59.52)	
Diagnostic tool				0.001
Cytology (%)	32 (48.48)	34 (51.52)	66 (16.54)	
Biopsy (%)	66 (32.51)	203 (67.49)	269 (67.41)	
Resection (%)	21 (32.81)	43 (67.19)	64 (16.05)	
Cytology (%)	32 (48.48)	34 (51.52)	66 (16.54)	
Non-cytology (%)	87 (26.12)	246 (73.88)	333 (83.46)	

Table 1-2. Patients demographics with *KRAS* mutation (n = 323 for *KRAS*)

Demographic parameters	KRAS Mu n = 28	KRAS WT n = 295	Total n = 323	*P* value
Age, yr (range)	65.96 (43-85)	65.50 (25-88)	65.60 (25-88)	
Sex (M:F)				
Male	22 (78.57)	199 (67.45)	221 (68.42)	
Female	6 (21.43)	96 (32.55)	102 (31.58)	
Smoking history				< 0.001
Current	10	112	122	
Ex-smoker	9	75	84	
Non-smoker	4	108	112	
Unknown	5	0	5	
Smoker	19 (67.85)	187 (63.38)	206 (63.77)	
Non-smoker	9 (32.15)	108 (36.62)	117 (36.23)	
Diagnostic tool				
Cytology (%)	4 (8.34)	44 (91.66)	48 (14.86)	
Biopsy (%)	21 (9.30)	205 (90.70)	226 (69.96)	
Resection (%)	3 (6.63)	46 (93.87)	49 (15.18)	
Cytology (%)	4 (8.34)	44 (91.66)	48 (14.86)	
Non-cytology (%)	24 (8.73)	251 (91.27)	275 (85.14)	

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specimens. The proportion of adenocarcinoma was highest in cytology; being 59 out of 66 cases (89.4%), followed by resection; 52 out of 64 cases (81.3%), and biopsy; 173 out of 269 cases (64.3%). Table 3 shows more detailed information about the diagnostic tools and their pathologic subtypes.

Table 2. Correlations between pathologic subtypes and *EGFR*, *KRAS* mutations

Pathologic types	EGFR Mu	EGFR WT	KRAS Mu	KRAS WT
Adenocarcinoma	111	170	23	189
SqCC	5	64	3	61
Large cell carcinoma		15		13
NSCLC	1	6	1	11
SCLC		12	1	11
Double primary(adeno + SqCC)	2	1		7
Pleomorphic carcinoma		2		1
Metastatic carcinoma		6		
Clear cell carcinoma		1		
Malignant melanoma		1		
Mucoepidermoid carcinoma		2		2
Adenoca.	113 (39.78)	171 (60.22)	23 (10.50)	196 (89.50)
Non-adenoca.	6 (6.96)	107 (93.04)	5 (4.80)	99 (95.20)
		$P < 0.001$		$P < 0.001$

KRAS mutations and clinicopathologic characteristics

Of the 323 patients who had received *KRAS* mutation test, 221 were male, and 102 were female (male:female = 68.4%:31.6%). The age of the patient ranged from 25 to 88 years old, with median age of 65.6 years. Similar to those of *EGFR* mutation tests, biopsy specimens were the most common, being 206 (63.8%). 49 (15.2%) and 48 (14.9%) samples were resection and cytology specimens, respectively.

A total of 28 of 323 patients were positive in *KRAS* mutation test (8.7%), and 22 were male, and 6 were female (male:female = 22 [78.6%]:6 [21.4%]). None of the 28 patients had *EGFR* mutation. Among the 28 *KRAS* mutation positive patients, 19 patients had smoking history (smoker:non-smoker = 19 [67.9%]:9 [32.1%]). Point mutations in codon 12 were most frequently observed mutation (18 cases, 5.6%), followed by mutation in codon 61 (7 cases, 2.2%). Mutation profiles of *KRAS* are summarized in Supplementary Table 1. Detection of *KRAS* mutation was highest in biopsy specimen; 21 of 226 (9.3%), followed by cytology; 4 of 48 (8.3%), and resection; 3 of 49 samples (6.6%). More information about patient data, smoking history and diagnostic tools of *KRAS* mutation are summarized in Table 1.

Table 3. Diagnostic tools of *EGFR* mutations and association with pathologic subtypes

Pathologic types	Cytology (n = 66)	Biopsy (n = 269)	Resection (n = 64)	Total (n = 399)	*P* value
Adeno	59	171	51	281 (70.42)	
SqCC	5	57	7	69 (17.29)	
Large cell carcinoma		12	3	15 (3.75)	
NSCLC	1	6		7 (1.75)	
SCLC	1	11		12 (3.07)	
Double primary (adeno + SqCC)		2	1	3 (0.75)	
Pleomorphic carcinoma		2		2 (0.50)	
Metastatic carcinoma		5	1	6 (1.50)	
Clear cell carcinoma		1		1 (0.25)	
Malignant melanoma		1		1 (0.25)	
Mucoepidermoid carcinoma		1	1	2 (0.50)	
Adenocarcinoma	59 (89.39)	173 (64.31)	52 (81.25)	281 (70.42)	
Non-adenocarcinoma	7 (10.61)	96 (35.69)	12 (18.75)	118 (29.58)	< 0.001

Table 4. Association of diagnostic tools of *KRAS* mutations and histopathologic subtypes

Pathologic types	Cytology (n = 48)	Biopsy (n = 226)	Resection (n = 49)	Total (n = 323)	*P* value
Adeno	39	133	40		
SqCC	5	63	4		
Large cell carcinoma		11	2		
NSCLC	3	2			
SCLC		12			
Double primary (adeno + SqCC)	1	4	2		
Pleomorphic carcinoma		1			
Metastatic carcinoma					
Clear cell carcinoma					
Malignant melanoma					
Mucoepidermoid carcinoma			1		
Adenocarcinoma	40 (83.33%)	137 (61.50%)	42 (85.71%)		
Non-adenoca	8 (16.67%)	89 (38.50%)	7 (14.29%)		< 0.001

Table 5-1. Mutation rates in non-smokers according to diagnostic tools

Smoking	Cytology		Biopsy		Resection	
	EGFR Mu (%)	EGFR WT (%)	EGFR Mu (%)	EGFR WT (%)	EGFR Mu (%)	EGFR WT (%)
Smoker	15 (46.87)	14 (51.85)	18 (30.50)	66 (58.92)	4 (20)	17 (54.83)
Non-smoker	17 (53.13)	9 (33.33)	37 (62.71)	38 (33.92)	16 (80)	12 (38.70)
Unknown	0	4 (14.82)	4 (6.79)	8 (7.16)	0	2 (6.47)
Total	32 (54.23)	27 (45.77)	59 (34.50)	112 (65.50)	20 (39.21)	31 (60.79)

Table 5-2. Mutation rates in non-smokers according to diagnostic tools

Smoking	Cytology		Biopsy		Resection	
	KRAS Mu (%)	KRAS WT (%)	KRAS Mu (%)	KRAS WT (%)	KRAS Mu (%)	KRAS WT (%)
Smoker	3 (75)	28 (63.63)	18 (85.71)	136 (66.34)	2 (66.66)	23 (50)
Non-smoker	1 (25)	16 (36.37)	3 (14.29)	69 (33.66)	1 (33.34)	23 (50)
Unknown	0	0	0	0	0	0
Total	4 (8.33)	44 (91.67)	21 (9.29)	205 (90.71)	3 (6.12)	46 (93.88)

The most common histologic subtype was adenocarcinoma (74.0%, 211 of 289 cases), followed by squamous cell carcinoma (22.1%, 64 cases), large cell carcinoma (4.5%, 13 cases), and small cell carcinoma (4.15%, 12 cases). Among 28 *KRAS* positive cases, 23 cases were adenocarcinoma, 3 were squamous cell carcinoma. Detection of adenocarcinoma was highest in resection specimen (85.7%, 42 of 49 cases). The association of diagnostic tools of *KRAS* mutation and histologic subtypes Table 4.

EGFR and *KRAS* mutations in non-smokers

It is well-known that *EGFR* mutation is more frequent in non-smoker, whereas *KRAS* mutation strongly correlates with smoking history. We investigated the correlation between the smoking history and mutation rates according to diagnostic tools. As expected, *EGFR* mutations were detected more frequently non-smokers in all three types of specimens (cytology, 53.1% [17 of all 32 mutation positive cases]; biopsy, 62.7% [37 of 59 cases]; resection, 80% [16 of 20 cases]). Details about the mutational status according to the diagnostic tools in non-smokers are summarized in Table 5.

DISCUSSION

Pyrosequencing is a non-electrophoretic, real-time, nucleotide extension sequencing method using luminometric detection (15-18). It is based on the transformation of pyrophosphate. Briefly, pyrophosphate is released when the nucleotide anneals to template and the primer extends. Subsequently, the released pyrophosphate is converted to adenosine triphosphate, which is utilized to produce light (15,17). Pyrosequencing is being increasingly used in various conditions, such as bacterial strain typing, mutation detection, SNP genotyping, and quantitative CpG island methylation analysis (15,19-24).

There are various and newly developed techniques for detecting mutations in lung cancer such as Amplification Refractory Mutation System (ARMS), cationic conjugated polymer-based fluorescence resonance energy transfer (CCP-RFLP), Smart Amplification Process (SmartAMP), pyrosequencing, etc (24). Standard method for detecting mutation of *EGFR* or *KRAS* is still direct DNA sequencing method. But, pyrosequencing has more advantages than conventional sequencing method, in that pyrosequencing is more sensitive, saves time, and cost-effective (11,15-18). Recently, some studies have been reported to show efficacy of detecting *EGFR* and *KRAS* mutations by pyrosequencing methods (11,17,25-27). However, most of these studies had performed only either one of *EGFR* or *KRAS* mutation testing by pyrosequencing, not both at the same time. Two previous reported studies have reported the usefulness of pyrosequencing in *EGFR*, *KRAS*, and *BRAF* mutations, but these studies were not solely done on lung carcinoma cases, and mainly focused on introducing new methodology with no available patient's clinical data (25,26).

The present study was performed with a large number of lung cancer patients (399 and 323 patients) in a single institution in Korea which shows both clinicopathologic characteristics and *EGFR* and *KRAS* mutation results by pyrosequencing. According to a nation-wide study of *EGFR* mutations in Korean patients, the overall *EGFR* mutation rate was 34.3% in patients with NSCLC and 43.3% in patients with adenocarcinoma (28). In our study, *EGFR* mutations were detected in 30.7% of NSCLC and in 39.8% of adenocarcinoma. Although, we did not compare pyrosequencing with other methods, pyrosequencing method is sensitive in detecting both *EGFR* and *KRAS* mutations. Similar to previous reports, *EGFR* mutations were prevalent in non-smokers (65.43%, $P < 0.001$), whereas *KRAS* mutations were common in smokers (67.85%, $P < 0.001$), and the two mutations are mutually exclusive in our study (6-8,25,29).

The utility of cytology samples in detecting mutations in lung cancer have recently been increased and relatively widely investigated (8,30-32). Cytology samples are especially useful in

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advanced lung cancer patients, when it is difficult to obtain tissue samples. In the present study, we compared the detection rates of both *EGFR* and *KRAS* mutations in cytology, biopsy and resection specimens. The detection rate of *EGFR* mutation in cytology was higher than non-cytology (biopsy or resection) materials (cytology: 48.48%, non-cytology: 26.12% [biopsy: 32.51%, resection: 32.81%]). Detection rate of KRAS mutation by cytology was comparable to non-cytology specimens as well (cytology: 8.34%, non-cytology: 8.73% [biopsy: 9.30%, resection: 6.63%]).

The present study showed that cytology specimens are comparable, or even better than non-cytology specimens, in detecting both *EGFR* and *KRAS* mutations. Cytology specimens are easier to obtain then tissue samples for several factors. First, lung biopsy is a very complicated process that requires both good patient condition and physician's technique. Many lung cancer patients have no specific symptoms until they develop into advanced stage, when the general condition of the patients is not good for lung biopsy. Moreover, in many cases, FFPE specimens are small, and contain many non-tumorous components, such as non-neoplastic lung parenchyma, fibrous tissue, or inflammatory cells, and could yield false negative results in mutation tests (8,33).

Detecting *EGFR* and *KRAS* mutations are crucial for treatment of non-small cell lung cancer patients, especially for advanced-stage patients who do not have many treatment options. In many cases, cytology specimens could be the only available samples for diagnostic or therapeutic approach. In conclusion, we suggest that cytology specimens are good alternatives that can readily substitute tissue samples for testing both *EGFR* and *KRAS* mutations. Moreover, pyrosequencing method is highly sensitive in detecting *EGFR* and *KRAS* mutations in lung cancer patients.

DISCLOSURE

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

AUTHOR CONTRIBUTION

Concept and design: Lee SE, Lee SY, Kim WS. Acquisition of data: Oh SY. Analysis and interpretation of data: Lee SE, Lee SY. Writing, review, and revision of manuscript: Lee SE, Lee SY, Park HK, Kim HJ, Lee KY. Study supervision: Kim WS. Approval of final draft: all authors.

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Supplementary Table 1. Profiles of *EGFR* and *KRAS* mutation in the type of specimens (n = 119 [*EGFR*], n = 28 [*KRAS*])

Mutation sites		Cytology	Biopsy	Resection	Total
EGFR mutation profile					
EGFR Exon 18	G719A		2		2
EGFR Exon 19	E746 _A750	13	25	10	48
	E746_P751insA		1		1
	E747_P753insP	1	1		2
	E747_P753insS		2		2
	E747_A753insS			1	1
	L747_P751insA	1	6		7
	L747_P753insS	1	6	4	11
	del E746 _S752insV	1			1
	L747_T751insP	2			2
	2239 T → C heterologous mutation	1	1		2
EGFR Exon 20	T790M		1		1
EGFR Exon 21	A859T	2	2		4
	L858R	10	19	6	35
Total		32 (26.89)	66 (55.46)	21 (17.65)	119 (100%)
KRAS mutation profile					
Codon 12	G12A		1		1
	G12C		3		3
	G12D	2	4	2	8
	G12V		6		6
Codon 13	G13D	1	2		3
Codon 61	Q61H	1	5		6
	Q61L		1		1
Total		4 (14.28)	22 (78.57)	2 (7.23)	28 (100%)