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EGFR and KRAS Oncogene Expressions Among Lung Cancer Patients in North Sumatera

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Abstract: ***Background: The urge in molecular alterations in cancer study showed new directions of lung cancer therapy to targeted therapy era. Nevertheless, there was a surge of resistance in targeted therapy due to double mutations in the oncogenic driver and the acquired resistance in T790M in EGFR. Unfortunately, there was still data limitations in epidemiological data in the expressions of EGFR and KRAS in Indonesia, particularly North Sumatera. Aims: The aim of this study was to determine the clinical characteristics of KRAS dan EGFR mutations in lung cancer patients in North Sumatera and whether there was an association between KRAS mutations and the therapy response of patients with and without EGFR-TKI. Methods: This is an observational study with retrospective cohort enrolled subjects diagnosed with lung cancer confirmed by histopathology examinations. EGFR and KRAS mutations were detected using sanger sequencing from the extractions of paraffin block using certain primers particularly in exon 2 of KRAS, exon 19, and exon 21 of EGFR. Therapy response was measured by RECIST criteria after 3 months of chemotherapy and targeted therapy. Results: Total 52 subjects had involved and completed all study procedure. There was none of exon 2 KRAS mutations and 6 subjects had EGFR mutations composed by 3 subjects had single mutations of exon 19, 1 subject had intron 190 mutations, and 2 subjects had double mutations. After 3 months of therapy, RECIST criteria was performed and showed no associations between EGFR mutations and RECIST with p-value: 0.08. Conclusions: KRAS mutations was not the cause of resistance in both systemic chemotherapy and EGFR-TKI in North Sumatera Populations.***

Keywords: KRAS, EGFR, lung cancer, RECIST, clinical characteristics

1. Introduction

The change in oncology directions to molecular alterations made targeted therapy becomes future in cancer treatment, particularly lung cancer. EGFR was the first oncogenes known and had substantial effect in increasing survival rate in non-small cell lung cancer (NSCLC) particularly adenocarcinoma that was the most common histology type of lung cancer. Yet, there is a surge of EGFR resistance that has been discussed in recent articles. KRAS mutations has been known as one of the most common etiology of EGFR resistance in patients diagnosed with lung cancer.

EGFR and KRAS was associated in RAS-RAF-MAPK pathway that begins after tied up the EGF in its receptor (HER2 dominated) and detached the phosphate element. The phosphate then bond with GRB-2 and SOS. RAS protein played a role as a switch of cellular proliferative signal by changed GDP to GTP. The mutations of RAS protein will result in the resistance of EGFR-TKI that has blocked HER2 receptor.

KRAS is the most common mutated protein in RAS protein. The mutation is predominantly occurred in exon 2 in codon 12 and codon 13, about 90%. KRAS mutation is related to current smoker or ex-smoker with higher tobacco consumptions. KRAS mutation also related to resistance of chemotherapy, radiotherapy, and targeted therapy.

The aim of this study was to determine the clinical characteristics of KRAS dan EGFR mutations in lung cancer patients in North Sumatera and whether there was an association between KRAS mutations and the therapy response of patients with and without EGFR-TKI.

2. Method

This is a cohort retrospective study held in Department of Pulmonology and Respiratory Medicine from 2017-2021. This study involved few oncology hospitals centers in North Sumatera including H. Adam Malik General Hospital, MurniTeguh Hospital, and Santa Elizabeth Hospital. Samples were histopathology preparations obtained from bronchoscopy, trans-thoracal lung biopsy, and thoracotomy. The inclusion criteria were lung cancer patients confirmed by clinical, radiological, and histopathology, age more than 17 years old, and had been administered minimal 3 cycles of systemic chemotherapy or 3 months of targeted therapy. The exclusion criteria were damage samples in preparations and examinations, failure to DNA extractions, and amount less than 50 tumor cells in a slice of paraffin block.

Consecutive sampling method was used to determine the subjects involved in this study. All the samples were matched the paraffin block and complete medical record. After that, all the paraffin blocks were re-examinations by two pathologists to ensure sufficient tumor cells in a slice of paraffin for molecular examinations. Then, the related

medical records were followed to evaluate the therapy response objectively by RECIST criteria.

EGFR mutations were examined by two methods. First method was sanger sequencing and ARMS PCR. KRAS mutation was examinated by sanger sequencing. PCR was performed with the MyTaq™ HS Red Mix protocol. Each PCR reaction contains 25 L of MyTaqTM HS Red Mix, 1 L of forward primer, 1 L of backward primer, 3 L of DNA template, and 20 L of ddH2O. EGFR primers matched Abdedian (1) while exon 21 primers were designed independently. ARMS PCR EGFR Exon 21 was performed according to Dahse et al. (2008) with modifications. While KRAS Exon 2 primers according to Zinsky et al. (1) . Details are attached in the following table.

Table 1: Primers used in the study for KRAS dan EGFR

Primer Name	Primer	Primer Sequencing (5' – 3')	Primer Concentrations (µM)	Amplicon Size (bp)
KRAS	Forward	AAGGCCTGCTGAAAATGACTG	20	173
	Reverse	CAAAGAATGGTCCTGCACCAG		
EGFR EXON 19	Forward	TCACTGGGCAGCATGTGGCA	20	241
	Reverse	CAGCTGCCAGACATGAGAAA		
EGFR EXON 21	Forward	AGAGCCTGGCATGAACATGA	20	370
	Reverse	CGAGCTCACCCAGAATGTCT		
EGFR 21 ARMS PCR	Forward (F)	AGGGTCTTCTCTGTTTCAGGGCAT	10	
	Reverse T (T)	TTCCGCACCCAGCAGTTTGGCTA		FT: 137
	Reverse G (G)	CGCACCCAGCAGTTTGGTTC		FG: 134

Sequencing results were imported, converted to reverse complement, and analyzed using ClustalW in BioEdit Sequence Alignment Editor 7.2.5 editions. The reverse sequence was also analyzed to human reference genome NG_007524.2 (LRG_433) using nBLAST NCBI using standard preferences. The BLASTn results were also aligned with correspond Coding Sequences/ (CDS). Lastly, the sequence was analyzed using FinchTv 1.4 Software by using reverse complement views.

Statistical analysis was performed using SPSS ver 24.0. Univariate analysis was described the epidemiological and clinical characteristics of the subjects while Chi Square Test was used to determine the associations between the KRAS and EGFR mutations and the response of therapy.

3. Results

Total 52 subjects enrolled and completed all study procedure. The majority of subjects were male, age > 60 years old, heavy smoker, and bataknese ethnic. Clinicopathological revealed most subjects were adenocarcinoma, had systemic therapy, but only 23% subjects had radiation therapy. Detailed characteristics were mentioned in Table 2.

Table 2: General characteristics of populations

No.	Characteristics	N	%
1	Sex		
	Female	12	25
	Male	40	75
2	Smoking Status		
	Neversmoker	7	13.5
	Light smoker	6	11.5
	HeavySmoker	39	75
3	Age		
	<40 yearsold	1	1.9
	40-60 yearsold	23	44.2
	>60 yearsold	28	53.8
4	Ethnics		
	Bataknese	32	61.5
	Javanese	11	21.2
	Minangnese	1	1.9
	Malay	5	9.6
	Tionghoa	3	5.8
5	Histopathology type		
	Adenocarcinoma	36	69.2
	SquamousCell	12	23.1
	SmallCellCarcinoma	4	7.7
5	Radiotherapy		
	No	40	76.9
	Yes	12	23.1
6	Treatment		
	Systemic Therapy	46	88.5
	Targeted Therapy	6	11.5

Further analysis in samples with EGFR mutations showed dominations of male (66%), age > 60 years old (83%), heavy smokers (66%) and adenocarcinoma (100%) but the statistical analysis showed no difference among clinical characteristics including gender, age, smoking status, and histopathology type and the EGFR mutations with p-value > 0.05.

KRAS sequence analysis

Almost all subjects had the expressions of RAS protein, yet none of subjects had the exon 2 of KRAS mutations. In all sequence analysis using Bioedit, BLASTn, and FinchTV revealed GGT GGC sequence of KRAS Exon 2 in the codon 12 and 13 were shown at base positions 219 to 224.

EGFR sequence analysis

PCR test showed all subjects had the EGF expressions while 3 subjects had exon 19 mutations, 1 subject had intron 19 mutations known as single nucleotide polymorphisms (SNP), and 2 subjects had double mutations for both exon 19 and exon 21. From sequence analysis showed there were few amino acid substitutions detect in the certain subjects with the majority of subjects showed changes in T – C in exon 19 and 21 while SNP showed 3 types of amino acid changed including T – C, C – T, and C – A in forward and reverse sequencing.

Therapy Response (RECIST) after 3 months

All subjects enrolled in this study had completed first RECIST after 3 months of targeted therapy and 3 cycles of chemotherapy. However, the impact of KRAS mutations in RECIST criteria cannot be assessed due to no mutations detected in this study. After 3 months of therapy, RECIST criteria was performed and showed no associations between EGFR mutations and RECIST with p-value > 0.05 (Table 3).

Table 3: The associations between EGFR mutations with RECIST 3 months

EGFR mutations	RECIST 3 months				p-value
	Complete response	Partial Response	Stable Disease	Progressive Disease	
Single mutations (exon 19)	1	1	0	1	0.08
Double mutations (exon 19 and exon 21)	0	1	0	1	
No mutations	0	6	11	19	

Fisher Exact Test

4. Discussions

The era of targeted therapy had given rise the increase of survival rate including good therapy response in lung cancer patients. Yet, there were few resistances incidence of EGFR-TKI in recent years. KRAS mutation has been known as the main co-incidence with EGFR and significantly related with poor response in lung cancer patients with targeted therapy. Further, the mutations of KRAS also related to poor prognosis in systemic treatment. In this study, we found no mutations of KRAS exon 2. Exon 2 was the highest prevalence of KRAS mutations among all type of KRAS mutations. But in this study, we found no mutations of exon 2 of KRAS. This study is the continuing study from Soeroso et al that showed no mutations of KRAS exon 2 in lung cancer patients. After added the additional sample, the results were still the same with no mutations found. This is the interesting findings because global world data showed KRAS mutations were founded approximately 20% in all lung cancer cases. Exact mechanisms of absence of KRAS mutations were still unknown but the environmental exposure and other oncogenes involvement may predispose this finding.

The mutations of EGFR in this study were lower than global data and Indonesian data that showed the mutations of EGFR was about 64% as general, 67% was common mutations, 19% was uncommon mutations, and 14% mix mutations. This study also showed different clinical characteristics of EGFR mutations compare with most of recent literatures that showed the majority of EGFR mutations were female and never smoker. Yet, this study revealed that most subjects with EGFR mutations were male, older age, and heavy smoker. This is also the interesting findings although the exact mechanisms caused this different epidemiology data was not clearly understood. Environmental and genetic alterations in certain populations including Bataknese ethnic as the predominant ethnic in North Sumatera may play a role as factors contribute to fewer molecular alterations in lung cancer pathogenesis. Recent study also stated that North Sumatera has lowest prevalence of oncogenic driver and also lower incidence of main enzymes mutations involved in nicotine metabolism that played a significant role in lung cancer pathogenesis.

This study also showed that there was no significant association between EGFR mutations and the therapy response of patients diagnosed with lung cancer. This is different from recent studies that showed significantly improve of survival rate and therapy response in patients with EGFR mutations and had EGFR TKI compare with systemic chemotherapy. The difference result might be resulted from fewer subjects with EGFR mutations treated with EGFR-TKI, so it is difficult to generalize the population. Further, smoking status also affect the response of therapy in patients with lung cancer. A study showed lower response rate of EGFR therapy in current smoker with heavy cigarette consumption. This is along with this study that showed the majority of subjects had EGFR-TKI were heavy smoker. Different molecular pattern in smoking related lung cancer might predisposed to this poor response. Unfortunately, this study cannot determine whether KRAS mutations as one of the most common causes of EGFR-TKI resistance and strongly related to lung carcinogenesis affect the lower response of therapy due to absence of KRAS mutations.

5. Conclusions

This study showed that no mutations of KRAS and lower incidence of EGFR mutations with different clinical characteristics compare with majority of studies. So, KRAS

mutations was not the cause of resistance in both systemic chemotherapy and EGFR-TKI in North Sumatera Populations. The more detailed molecular mapping of genetic alterations is needed in North Sumatera populations to determine whether it has clinical impact in lung cancer patients.