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Our Results of Genetic Mutation Analysis in Lung Cancer

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Abstract

Objective: Molecular pathways thought to be effective in carcinogenesis in non-small cell lung cancer (NSCLC), new agents have developed in cancer cells against specific targets on these molecular pathways. We wanted to determine the genetic mutation analyzes of our patients before treatment begins and to see the genetic mutation data of our country. **Materials and Methods:** The pathology results of 680 patients with NSCLC were evaluated (between 2015-2017). Mutation detection and EGFR mutation analysis were performed by real time PCR method. For gene translocation detection: by using specific a probe to Anaplastic Lymphoma Kinase (ALK) and ROS1 gene molecules, fluorescence in situ hybridization (FISH), ALK and ROS1 gene rearrangement tests were performed. **Results:** 542 patients had adenocarcinoma (79.7%), and 138 patients (21.3%) had non-adeno associated NSCLC pathology. The EGFR mutation was found in 651 patients, 75 (11.5%) were positive and were mutant. ALK was found to be positive in 11 patients (2.25%) in 488 patients. ROS was evaluated in 393 patients and in 4 patients (1.01%) it was evaluated as positive. **Conclusion**: The most common mutations for adenocarcinoma occur with EGFR, ALK, and ROS 1 gene rearrangements. Unlike literature data, we found that 3 mutations were higher than the literature in terms of age and smoking rates were higher in cases with EGFR mutation. Genomic examination should be performed in non-adeno NSCLC, especially in non-smokers. With high number of cases and having a mosaic of the country, our study is important to share.

Keywords: cancer, treatment, genetic mutation.

Introduction

Although the current classification of lung cancer, which is the most common cause of cancerrelateddeaths, is based on small-cell lung cancer and non-small cell lung cancer, since the early 2000s, molecular pathways thought to be effective in carcinogenesis in non-small cell lung cancer (NSCLC) and new agents have begun to be developed in cancer cells against specific targets on these molecular pathways.

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The most effective biomarker for targeted therapy in advanced NSCLC is changes in somatic genes called "driver mutations". These mutations occur in genes that encode proteins that play a role in the growth and death of the cell. Drive mutations cause transformation in normal cells, causing the cell to acquire malignant character. In particular, molecular changes such as epidermal growth factor receptor (EGFR), anaplastic lymphoma kinase (ALK), and "protooncogenictyrosine-protein kinase 1 ROS" (ROS1) have highly effective pharmacological forms in the targeted treatment process, and they have opened a new era in the treatment of lung cancer.

In our study, we thought that starting out by setting the target initially would have a positive effect on treatment planning and with a multidisciplinary approach; we wanted to determine the genetic mutation analyzes of our patients before treatment begins and we aimed to see how the genetic mutation data of our

country (by being both in Asia and in Europe) is in relation with the world data[1-3].

Materials and Methods

The pathology results of 680 patients with NSCLC who had referred in 2015-2017 to the University of Health Sciences, Dr. LütfiKirdarKartal Training and Research Hospital, Department of Radiation Oncology were evaluated.

SPSS program was used for statistical data analysis. Mutation detection and EGFR mutation analysis were performed by real time PCR method.

For gene translocation detection: by using specific a probe to Anaplastic Lymphoma Kinase (ALK) and ROS1 gene molecules, fluorescence in situ hybridization (FISH), ALK and ROS1 gene rearrangement tests were performed.

Results

542 patients had adenocarcinoma (79.7%), and 138 patients (21.3%) had non-adeno associated NSCLC pathology. The median age was 63 (34-88) years. 538 of the cases were male (79.1%) and 142 (20.9%) were female. In pathological examination; 348 patients were examined from lung (51.2%), 66 (9.7%) from mediastinal lymph nodes, 22 from pleura (3.2%) and others from metastatic sites (35.9%). The EGFR mutation was found in 651 patients, 75 (11.5%) were positive and were mutant.

Table-1: EGFR mutations

ALK was found to be positive in 11 patients (2.25%) in 488 patients.

Table-2: ALK mutations

ROS was evaluated in 393patients and in 4 patients (1.01%) it was evaluated as positive.

Table-3: ROS mutations

In our study: Exon 19 deletion mutation was found as 49% in 37 patients, Exon 21 L858R mutation was found as 27% in 20 patients, Exon 20 in-frame mutation was found as 7% in 5 patients, G719 Point mutations in Exon 18 was found as 11% in 8 patients and S768I mutation in Exon 20 was found as 5% in 4 patients.

Table-1: EGFR mutations

	EGFR mutations			
	Total	**Present**	**Absent**	**P value**
Age	652			0,016
60>	248	19	229	
60≤	404	56	348	
Gender	652			0,001>
Male	516	31	485	
Female	136	44	92	
Smoking Status	652			0,003
Smokers	460	64	396	
Non-Smokers	192	11	181	
Histology	652			0,001>
Adenocarcinoma	520	72	448	
Nonadenocarcinoma	132	3	129	

Table-2: ALK mutations

	ALK mutations			
	Total	**Present**	**Absent**	**P value**
Age	488			0,76
60>	188	5	183	
60≤	300	6	294	
Gender	488			0,13
Male	396	7	389	
Female	92	4	88	
Smoking Status	488			0,001>

Smokers	366	2	364	
Non-Smokers	122	9	113	
Histology	488			0,47
Adenocarcinoma	389	8	381	
Nonadenocarcinoma	99	3	96	

Table-3: ROS mutations

	ROS mutations			
	Total	**Present**	**Absent**	**P value**
Age	393			0,65
60>	156	2	154	
60≤	237	2	235	
Gender	396			1
Male	309	3	306	
Female	84	1	83	
Smoking Status	393			0,206
Smokers	308	2	306	
Non-Smokers	85	2	83	
Histology	393			0,035
Adenocarcinoma	306	1	305	
Nonadenocarcinoma	87	3	84	

Conclusion

The most common mutations for adenocarcinoma occur with KRAS, EGFR, ALK, and ROS 1 gene rearrangements[1-3]. However, since the clinical efficacy of targeted therapies for KRAS mutation has not been proven, we did not inclued this mutation analysis to our study[4].

The second most common mutation is the EGFR mutation, located at position 12 of the short arm of chromosome 7. Prevalence ranges from 10-15% in whites and 30-50% in Asians [5-7]. In our study, similar proportions of EGFR was studied in 651 patients; 75(11.5%) were positive and these were mutant type. This mutation is often found in the adenocancer histology of non smoking female patients [7-9]. When the cases that were positive in our study were examined, it was determined that the average age was 66.7 and it was found to be higher in terms of sex at women with a statistically significant level. However, unlike the literature, this mutation wassurprisingly found more frequent in smokers that smoke more than 10 packets/year ($p < 0.01$).

EGFR mutations typically occur in the receptor tyrosine kinase domain, which activates signaling and enhances susceptibility to EGFR-TKI such as gefitinab, erlotinib. The presence of EGFR tyrosine kinase mutations in advanced NSCLC is a good marker for EGFR-TKI sensitivity as well as a better prognosis [10-11]. EGFR-TKI response rates in EGFR mutated NSCLC patients are at least twice as high as those obtained with conventional chemotherapy [12-13]. Two common EGFR sensitization mutations identified are the in-frame deletion in exon 19, which accounts for 45% of EGFR mutations in lung cancer, and the L858R point mutation in exon 21, which accounts for 40% of EGFR mutations. The third most common type is in-frame insertions in exon 20 and is detected between 5-13%. The others include point mutations in exon 18 at G719(3%), mutations in exon 21 at L861Q (2%) and in-frame insertions in exon 19 (1%) [7,14-16].

In our study, exon 19 deletion mutation rate was 49% in 37 patients, exon 21 L858R mutation was 27% in 20 patients, exon 20 in-frame mutation was 7% in 5 patients, point mutations in exon 18 at G719 was 11% in 8 patients and S768I mutation in exon 20 was 5% in 4 patients.

Another mutation, ALK, is located at position 23 of the short arm of the 2nd chromosome [17]. It is detected between 3-7% in both white and Asian populations of NSCLC cases [18,19]. In our study, ALK mutation could be studied in 488 patients and found positive in 11 patients (2.25%), which is lower than the literature results. The most common ALK fusion partner detected in NSCLC cases is the echinoderm microtubule associated protein-like 4 (EML4) gene. EML4- ALK fusion can bee observed in; young patients who have never smoked, or mild smokers under 10 packs/year, and acinar or histological signet

ring adenocarcinoma [20,21]. In our study, the mean age of the positive cases was 59.8, and similar to the literature, there was statistically significant ALK positivity was detected in non-smoking cases. Clinical trials have shown significant improvement in response rate and progression-free survival in patients with NSCLC who were detected as crizotinib ALK-positive, when compared with standard chemotherapy [22,23]. The novel order or fusion proteins of ALK gene in tumor cells can be detected by fluorescence in situ hybridization (FISH), immunohistochemistry (IHC) and reverse transcription polymerase chain reaction of cDNA (RT-PCR). The FISH method is the gold standard for the identification of patients with ALK-positive NSCLC. Many multiple monoclonal antibodies have been developed which can be used in the IHC method. Although it can be considered as a screening method to identify ALK positivity, positive results need to be confirmed by FISH method [24]. So we have also used these valid methods in our study.

Another mutation we have examined in our study was ROS1 proto oncogene, which locates at the 6. chromosome in position 22. ROS 1 rearrangement leads to the formation of active fusion proteins and is observed in 1-2% of NSCLC cases [25,26]. Crizotinib showed evidence of in vitro activity and early clinical activity in ROS1-rearrangemented NSCLC [27].

In our study, ROS1 mutation was evaluated in 393 patients and in 4 patients (1.01%), it was positive, this result is compatible with the literature. ROS 1 rearrangement is associated with adenocarcinoma and is commonly detected in patients younger than 50 years old who are non smokers or mild smokers, and this mutation is not associated with EGFR, ALK mutation [25].

In our study, the mean age of positive cases was 53.8 and it was thought that the result was not statistically significant in terms of smoking but it was thought that the low number of cases could lead to this result. In addition, consistent with the literature, EGFR and ALK mutations were found to be negative in ROS 1 positive cases.

All of these mutations are present in adenocancer histology as well as in other subtypes.

For this reason, in our study, out of 138 patients who were found to have NSCLC other than adenocancer; EGFR was positive in 2(1.4%), ALK was positive in 3(3%) and ROS was positive in 3(3%) patients. In accordance with the literature, these cases consisted of non-smokers. For this reason, genomic examination should be performed in non-adeno NSCLC, especially in nonsmokers.

The most important limitations of our study are the inability to perform the staging and not to study the mutations according to the metastases. Since our study has aimed to examine the epidemiologic mutation in our country, no evaluation was made in terms of survey.

However, we believe that our study is an important work in terms of high number of cases investigated and so that our hospital is located in İstanbul, the study may show the entire mosaic of Turkey by being at the center of the country.

Discussion

In our study that evaluated 680 NSCLC patients, As, we aim to share the data of Turkey (by being in the middle of Asia and Europe) in terms of genetic mutations. EGFR mutation frequency in Western populations is 10-15%, while in Asian populations this rate is 30-50%. Similarly to Europe, we have a rate of 11.2. In the literature, ROS1 positivity was found to be 1-2% and ALK positivity was found to be 3-7%. In our study, it was 1.01% for ROS1 and 2.25% for ALK. Interestingly, unlike literature data, we found that 3 mutations were higher than the literature in terms of age and smoking rates were higher in cases with EGFR mutation.

EGFR mutation, ALK and ROS1 rearrangements are the best-defined molecular changes in NSCLC and have a target role in therapy. These genomic changes should be looked at in all patients with adenocarcinoma or NSCLC pathology in order to determine the target from the beginning and avoid unnecessary treatment.

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