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Evaluation of Common Mutations in Exon 2 and 3 of the K-ras Gene in Patients with Lung Cancer

Mohammad Ali Saremi^{1,*}¹ Personalized Medicine Research Center of AmittisGen, Tehran, Iran* Corresponding author: Mohammad Ali Saremi, Personalized Medicine Research Center of AmittisGen, Tehran, Iran. E-mail: masaremi@yahoo.com

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Abstract

Lung cancer is the deadliest cancer in Iran after gastric cancer. The vast majority (85%) of cases of lung cancer are due to long-term tobacco smoking. About 10–15% of cases occur in people who have never smoked. These cases are often caused by a combination of genetic and environmental factors. Many human cancers are the result of mutations in the RAS family, and lung cancer is no exception. In this study, mutations in codon 12 and 13 of exon two were performed in 50 lung tumors from the Iranian Institute of Oncology. The exon 2 of the gene was amplified by PCR and sequenced for detection of the point mutation in codon 12 and 13. Of the 50 samples, 13 had mutations in codon 12 and 13, of which only two patients had single mutations in codon 12. No significant relationship was not found between age ($P = 0.43$) and gender ($P = 0.37$) and mutations in this gene. No significant relationship was found between disease stage and mutation in this gene ($P = 0.51$). Identifying k-ras gene mutations as an oncogene and having an effect on the treatment process can help the physician to choose the appropriate treatment.

INTRODUCTION

Lung cancer is the deadliest cancer in Iran after gastric cancer (1). In the US, 27 percent of all deaths from lung cancer are due to lung cancer. Studies have estimated that 7 percent of people will get lung cancer during their lifetime, and 6 percent of them will die (2). Most cancers that start in the lung, known as primary lung cancers, are carcinomas. The two main types are small-cell lung carcinoma (SCLC) and non-small-cell lung carcinoma (NSCLC). The most common symptoms are coughing (including coughing up blood), weight loss, shortness of breath, and chest pains. The vast majority (85%) of cases of lung cancer are due to long-term tobacco smoking. About 10–15% of cases occur in people who have never smoked (3). These cases are often caused by a combination of genetic factors and exposure to radon gas, asbestos, second-hand smoke, or other forms of air pollution. Like all genetic diseases, cancer results from changes in DNA (4). Tumor cell DNA has many variations from point mutations to extensive chromosomal abnormalities such as deletions and translocations. Genetically, two types of genes are involved in the development of cancers, including oncogenes and tumor suppressor genes (5). With the advances in molecular biology in recent decades, we have been able to identify the changes in the DNA sequence of cancer cells and provide targeted therapies. Epidermal growth factor receptor (EGFR) is a tyrosine

kinase receptor that belongs to the ErbB family and plays an essential role in tumor progression (6). The use of tyrosine kinase inhibitors that block the ErbB message delivery pathway is one of the relevant clinical advances in the field of targeted cancer treatment. Increased expression of epidermal growth factor receptors and its ligand has been reported in many epithelial tumors (7). In recent years, it has been shown that tyrosine kinase inhibitors that target ATP, an epidermal growth factor receptor, may have antitumor activity. Studies have shown that treatment with epithelial growth receptor inhibitors should continue until the tumor size is not increased, even if no significant change in gene expression is observed (8). Many human cancers are the result of mutations in the RAS family, and lung cancer is no exception (9). One member of this family is the k-ras proto-oncogene, which is located on the long arm of chromosome 12 and encodes the 21-kDa protein. It is a member of the GTPase family that binds to the cell membrane and converts extracellular messages into intracellular messages via membrane receptors such as EGFR, which induces proteins required for receptor activity such as PI3K (10). The most common hotspot k-ras gene mutations are in exons 2, 3 and 4 of this gene (11). Mutations in this gene are associated with low patient survival and increased lung cancer metastasis, and

patients with standard anti-EGFR therapy do not respond. 97% of the k-ras somatic mutations are in exon two of this gene (12). In this study, we examined the frequent mutations in exon two and three of the k-ras gene in patients with lung cancer and compared the results with clinical and pathological manifestations.

MATERIALS AND METHODS

In this study, mutations in codon 12 and 13 of exon two were performed in 20 freezer lung tumor specimens prepared from the Iranian Institute of Oncology and 30 paraffin blocks. The samples were then transferred to the laboratory of the Personal Medical Research Center by cold chain preservation and stored in the freezer-80 until extraction. For DNA extraction, paraffin-

embedded tissues were the first deparaffinization by xylene, then similar to fresh tissues were extracted according to Tissue Genomic DNA extraction Favorgen Biotech protocol. The quality and quantity of DNA obtained were determined by agarose gel electrophoresis (Figure 1) and nano-drape. The PCR reaction was performed with specific primers for sequencing, according to Table 1. The PCR product was run on 1.5% agarose gel, and the results were observed in Figure 2. The PCR product was sequenced by ABI Genetic Analyzer 3500, reads were analyzed by chromas software, and genotypes of codons 12 and 13 were identified in the samples. Statistical analysis was performed by SPSS software, and a p-value of less than 0.05 was considered significant.

Table 1. Primer Sequence

Primer name	Sequence	Annealing Tm	Product size
K-ras-F	TGTGACATGTTCTAATATAGTCAC	52c	270bp
K-ras-R	ATTATCTTGTAATAAGTACTCATG		

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TGTGACATGTTCTAATATAGTCACATTTTCATTATTTTTATTATAAGGCCTGCTGAAAATGA
CTGAATATAAACTTGTGGTAGTTGGAGCTGGTGGC GTAGGCAAGAGTGCCTTGACGATA
CAGCTAATTCAGAATCATTTTGTGGACGAATATGATCAACAATAGAGGTAATCTTGTTTT
AATATGCATATTACTGGTGCAGGACCATTCTTTGATACAGATAAAGGTTTCTCTGACCATT
TCATGAGTACTTATTACAAGATAAT
  
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Codon 12 Codon 13

Figure 1. DNA Genomic

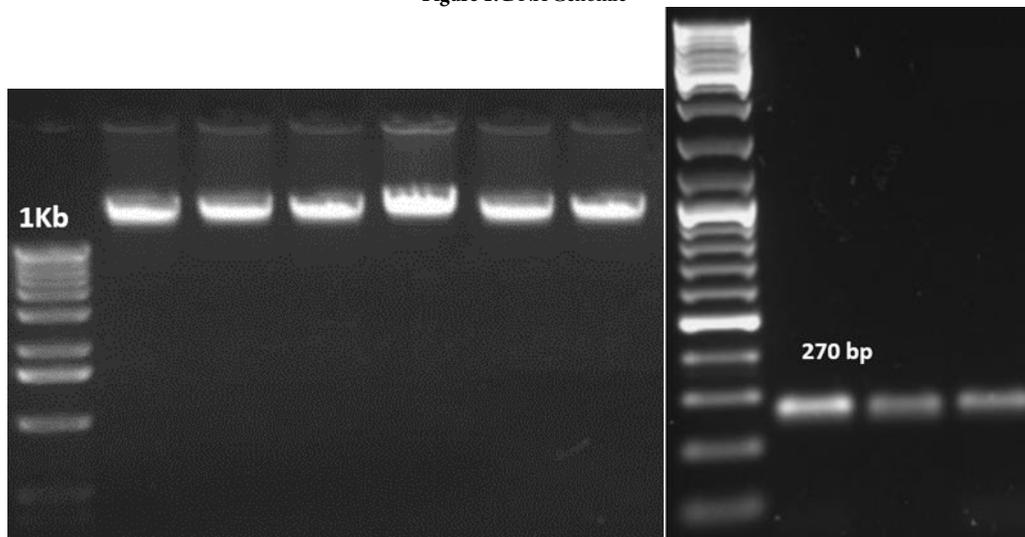


Figure 2. PCR Product

RESULTS

The study population consisted of 31 (62%) men and 19 (38%) women with a mean age of 59.7. Of the 50 samples, 13 had mutations in codon 12 and 13, of which only two patients had single mutations in codon 12. No significant relationship was not found between age ($P = 0.43$) and gender ($P = 0.37$) and mutations in this gene.

No significant relationship was found between disease stage and mutation in this gene ($P = 0.51$).

DISCUSSION

In this study, we evaluated the mutations in codon 12 and 13 in exon 2 of the k-ras gene in 50 patients with lung cancer. Of the 50 tumor samples, only 13 had mutations in these two codons. 2 cases had only

mutations in codon 12, and 11 had mutations in both codons 12 and 13. No association was found between age, sex, stage of the disease, and the incidence of mutation. Investigation of k-ras gene mutations is essential in the choice of therapeutic approach and, in particular, the use of tyrosine kinase inhibitor (TKI) drugs. In a study by Saleh Jazi et al. on 52 colorectal cancer tumor samples, 15.4% of the samples had a heterozygous mutation, and 74.6% of the samples had a wild genotype (13). Similar to our study, this group did not report any significant association between clinicopathological features and mutations in the k-ras gene. In another study, Zarifian Yeganeh et al. examined the rate of k-ras mutations in codon 12 and 13 exons 2 of 59 squamous cell carcinoma samples of the head and neck (14). The results showed that 17.5% of the samples had mutations at codon 12 and 13, of which the highest mutation was related to the conversion of glycine to aspartate at codon 13. In 2016, Zheng et al. examined the K-ras gene mutations in 1368 patients with lung adenocarcinoma (15). In study 113 (8.3%), the person had K-ras mutations at codons 12, 13, and 61. Accurate knowledge of the genetic profile of people with lung cancer is crucial in choosing the appropriate treatment approach, identifying k-ras gene mutations as an oncogene and having an effect on the treatment process can help the physician to choose the appropriate treatment. In this study, we found that this gene can be used as a predictor of treatment choice.

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