http://pmjournal.ir Review Article

Spring 2024, Volume 9, Issue 33 (14- 28)

Targeting Key Genes in the Early Diagnosis and Treatment of Lung Cancer with a Focus on Personalized Medicine: a Review Article

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DOI: 10.22034/pmj.2024.713765

Submitted: 2024-02-12
Accepted: 2024-05-19

Keywords:
Lung cancer
Key genes
Clinical biomarkers
Early diagnosis
Personalized medicine

How to Cite this Article:

M. Pezeshki, A. Ardalan, M. Nakhaee, S. Ziaei, R. Valipoor, E. Tamjidi, R. Naseri,"Targeting Key Genes in the Early Diagnosis and Treatment of Lung Cancer with a Focus on Personalized Medicine: a Review Article" Personalized Medicine Journal, Vol. 9, no. 33, pp. 14-28.

Abstract:
IntroductionLung cancer is the prevailing form of cancer globally, with a significant fatality rate among both males and females. Lung cancer is the third most frequent type of cancer in Iran, and it is becoming more common all the time. Patients are frequently diagnosed in the advanced stages of the disease, which contributes to the high death rate. Therefore, the ability to identify molecular markers is essential for both early diagnosis and the choice of conventional treatment for lung cancer. Numerous genetic variations have been found to be strongly linked to the development of lung cancer, according to studies. The aim of this work is to look into the genes that contribute to the development of lung cancer.
Materials and methods: The present review was authored using search terms related to lung cancer, key genes, clinical biomarkers, and early diagnosis that were found on PubMed, NCBI, Scopus, Science Direct, and Google Scholar.
Findings: Since the *EGFR, KRAS, BRAF*, and *TP53* genes are the most significant and involved in the development of lung cancer, finding mutations in these genes can be a valuable clinical diagnostic for lung cancer diagnosis and therapy.
Discussion and conclusion: With an emphasis on personalized medicine, the identification of genes linked to lung cancer may be utilized as clinical biomarkers for the disease's early diagnosis and effective treatment. The state of targeted lung cancer therapy and early detection techniques may be enhanced by molecular biomarkers. In the field of personalized medicine, identifying the genes linked to lung cancer as clinical biomarkers for early diagnosis and assessing treatment response to select a targeted treatment can be crucial in streamlining the therapeutic process, improving treatment response, lowering mortality, and lessening the material and spiritual harm this illness causes.

INTRODUCTION

Cancer is known as one of the main causes of death in economically developed and developing countries. About 12.7 million new cancer cases and 6.7 million cancer deaths occur annually (1). Most of the factors that cause cancer are among the factors that lead to DNA sequence changes or mutations (2). Lung cancer or lung carcinoma is a malignant lung tumor that can be identified by uncontrolled cell growth in lung tissues (3, 4). The incidence and mortality rate of lung cancer has increased significantly around the world (5). Lung cancer is a complex disease caused by

genetic and environmental factors derived from the interaction between these two factors. Lung cancer is the main cause of death due to cancer worldwide so that approximately 1.6 million people die from lung cancer every year (4, 6, 7). Studies have shown that about 8% of lung cancer cases are due to hereditary factors (8). Lung cancer is the second most common cancer in the Western Hemisphere (9). The highest incidence of lung cancer is in North America, Europe and East Asia. The incidence rate of this cancer is lower in Africa and South Asia (4). Lung cancer mortality is higher in developed countries than in less developed countries and is higher in men than women (9). In Iran, lung cancer ranks 7th in men and 10th in women, and it is the second and third leading cause of cancer death among men and women, respectively (10). The incidence of this cancer in Iran is increasing day by day (11). It has been estimated that more than 13% of newly diagnosed cases of cancer and approximately 27% of total cancer deaths are related to lung cancer (12). Despite the diagnostic advances in recent years, most of the cases related to lung cancer are diagnosed in advanced stages, and for this reason, the mortality rate is high (12-14). Lung cancer is of great importance among cancers due to its high prevalence of lethality and low 5-year survival rate (16, 15). Lung cancer is a complex pathological process that is divided into two main groups: small-cell lung cancer and non-small cell lung cancer (18,17). Non-small cell lung cancer is the most common tissue type of lung cancer with a percentage of about 85% (19). Non-small cell lung cancer includes three subgroups: adenocarcinoma, squamous cell carcinoma, and large cell carcinoma (4). Lung adenocarcinoma, which occurs on the surface of the lung tissue (4), accounts for most cases of lung cancer and its incidence rate is increasing (20). Several risk factors are involved in the occurrence of lung cancer. Among the known risk factors related to the increased risk of lung cancer, smoking has the strongest association, so it is reported in 85% of lung cancer cases (8). Cigarette smoke contains at least 73 known carcinogens, including benzopyrene. Cigarette smoke inhalation is one of the risk factors for this cancer in non-smokers. Such people usually live or work next to smokers (4). Another risk factor for lung cancer is radon gas. Radon gas is a colorless and odorless gas that is produced through the decay of the radioactive element radium and can cause lung cancer by causing mutations in the genome (21). Radon is the second most common cause of lung cancer in America, which is the cause of death of 21,000 people every year (4). Air pollution caused by burning wood, charcoal or other agricultural products for cooking and heat production is another factor of environmental risk (22). Women who are exposed to coal smoke have twice the risk of contracting it (23). Asbestos is another risk factor that can increase the risk of lung cancer. Smoking and being exposed to asbestos have an aggravating effect on the occurrence of lung cancer. In smokers who work with asbestos, the risk of developing lung cancer is 45 times that of the general population. Genetic factors and age also play an important role in the occurrence of lung cancer. Increasing age increases the risk of lung cancer by increasing the number of mutations in the genome. About 8% of lung cancers are hereditary. In general, the risk of lung cancer increases more than 2 times in people who have first-degree relatives with the disease (4, 24). Other environmental factors include metals such as Aluminium and Aluminium products, cadmium and cadmium compounds, chromium, beryllium and beryllium compounds, iron, steel, nickel-containing compounds, arsenic, mineral arsenic compounds, hematite extracted from underground sources, and combustion products. including the incomplete combustion of coal and the gases resulting from its burning, the smoke resulting from the burning of vehicle fuel, ionizing radiation including X-rays, gamma and plutonium, toxic gases such as methyl ether, bis ether, sulfur-containing gases, and crystalline silica dust (25). Among the risk factors, the genetic factor is considered as the main factor. This study aims to explore the significance of important genes in the development of lung cancer. Determining the genes linked to the development of lung cancer can be crucial to comprehending the lung's carcinogenesis process, facilitating early detection, and enhancing patient care. Lung cancer develops similarly to other cancers in terms of how it spreads. Put another way, the activation of oncogenes or the inactivation of tumor suppressor genes is the initial step towards lung cancer. (4). Changes in these two categories of genes along with changes in DNA repair genes such as the occurrence of mutations and single nucleotide polymorphisms (26) along with other changes in the genome such as deletions, integrations, chromosomal translocations, inversions and epigenetic changes such as changes in DNA methylation. Changes in the tail region of histones or changes in the regulation of microRNAs, all by affecting the inactivation of tumor suppressor genes and the activation of proto-oncogenes, can ultimately lead to the occurrence of lung cancer (4). Therefore, investigating and identifying the genes involved in lung cancer can play a major role in identifying the carcinogenesis process of this disease (12, 27, 28, 29). The most common genetic changes in the genome that can affect the incidence of lung cancer are single nucleotide polymorphisms at the genome level. Polymorphisms are naturally present in human genomic DNA and are used to identify individual differences related to

susceptibility to diseases (30). The presence of these polymorphisms in proto-oncogenes, tumor suppressor genes and DNA repair genes respectively lead to the creation of oncogenes (increased expression of a gene), the lack of function of the protein resulting from tumor suppressor genes and the loss or reduction of repair capacity in DNA repair genes. It leads to lung cancer (7, 15, 27). Therefore, the presence of this polymorphism in the mentioned genes can affect the risk of lung cancer (31). Recent studies to investigate the variants of the genomic range related to lung cancer significantly show that polymorphism changes in the regions of 15q25.1 (CHRNA5/CHRNA3/CHRNB4), 5p15.33 (TERT/CLPTM1L), 6p21.33 (BAT3/MSH5), 22q12 (CHEK2), 15q15.2 (TP53BP1) and 12p13 (RAD52) are effective in the risk of lung cancer (31-33). There are other susceptible regions in chromosomal positions 13q12.12, 3q28, 6q23-25, 18p11.23, 2p22.2, 14q13.1, 16p13, 20q13.11, 1q42-43, 7p12.1-12.3, and 7q31.3. Various studies have shown a significant association with the risk of lung cancer (12, 34-36). The mentioned regions related to lung cancer contain genes that are in three general categories proto-oncogenes, tumor suppressor genes and repair genes (2, 4). The presence of a polymorphism on a proto-oncogene can lead to an increase in product production and its transformation into an oncogene and is related to the risk of lung cancer (8). The presence of polymorphisms in tumor suppressor genes can lead to the production of inefficient proteins and is associated with the risk of lung cancer (37). On the other hand, the placement of polymorphism on DNA repair genes can also lead to a change (decrease) in DNA repair capacity and is associated with the risk of lung cancer (38). Mutations in two groups of proto-oncogene genes and tumor suppressor genes are the starting point of the carcinogenic process in the lung. The occurrence of mutation in proto-oncogenes causes excessive production of the product of a protoncogene and turns it into a cancerous gene that causes cancer. The occurrence of mutation in tumor suppressor genes causes the loss of function of these genes and can lead to the occurrence of lung cancer (2, 4, 28). Various studies have been conducted, and about 50 tumor suppressor genes and 100 oncogenes that play a role in the cell cycle and the incidence of lung cancer have been identified and investigated (39). Identification of mutations in genes that have the greatest effect on the occurrence of lung cancer can play an important role in identifying biomarkers for early diagnosis, reducing the rate of this cancer and also reducing material and spiritual damages (40, 41). Mutations in cancer genes and tumor suppressor genes are necessary to cause lung cancer. Identification of mutations in these two groups of genes can play a major role in lung cancer screening (prognosis and prediction) as molecular markers.

MATERIALS AND METHODS

In this research, to write this review article, the keywords of lung cancer, clinical biomarkers, key genes, and early diagnosis were searched in PubMed, NCBI, Scopus, Science Direct and Google Scholar websites, and after extracting related sources, the present article was based on They were written. Our goal in this study is to investigate the genes involved in the occurrence of lung cancer in such a way that after identifying and listing the genes involved in the occurrence of lung cancer, we investigated the genes that have the highest mutation rate and then the names of the common mutations. We examine the damaging process of mutations and its importance in the field of early diagnosis and treatment of lung cancer.

FINDINGS

Several oncogenes and tumor suppressor genes have been identified whose mutations are associated with lung cancer (42). *KRAS* gene, which is located at chromosomal position 12p12.1, is a member of the RAS family and one of the first oncogenes involved in lung cancer. Activating mutations in the *KRAS* oncogene are the most common oncogenic changes in lung adenocarcinoma, occurring in up to 40% of cases (42, 43). The second most important gene in the occurrence of lung cancer is the *EGFR* oncogene, which is located at chromosomal position 7p12.1-12.3 (44). Mutations in this gene lead to lung cancer by increasing its expression. Increased expression of this gene due to mutation has been observed in 43-89% of lung cancer cases. More than 90% of point mutations and deletions occur in exon 19 of this gene (42). The most important tumor suppressor gene involved in the occurrence of lung cancer is *TP53*, which is located in the chromosomal position 17p13 and plays an important role in preventing the occurrence of lung cancer. Mutations in this gene can cause lung cancer, as mutations in this gene have been reported in 50-60% of lung cancer cases (42, 45). Among other genes involved in the occurrence of lung cancer is the *BRAF* oncogene at chromosomal position 7q34, which is a member of the RAS family, and mutations in this gene have been reported in up to 8% of lung cancer cases. The hot spot of this gene for the occurrence of mutation is codon 600 of this gene (42, 46). Among other genes involved in the occurrence of lung cancer, we can mention the *HER2* gene, the mutations in exon 20 of this gene have been reported in about 2 to 4 percent of lung cancer cases. *EML4* and *ALK* genes are among the other effective genes in the occurrence of lung cancer, and changes in these genes have been reported in about 3 to 7 percent of lung cancer cases. Proto-oncogene ROS1, which is located at chromosomal location 6p22, is associated with lung

Table1. Names and frequencies of genes involved in the occurrence of lung cancer (2).

Gene	Cancer type	Genetic Alteration	Frequency%
EGFR	Adenocarcinoma	Point mutations and copy number variants	30-40
KRAS	Adenocarcinoma	Point mutations	20-30
MET	Adenocarcinoma	Slippage mutations, increased expression	2-5
ALK	Adenocarcinoma	Integration	3-7
BRAF	Adenocarcinoma	Point mutations	۰.05-5
ROS1	Adenocarcinoma	Integration	2-3
RET	Adenocarcinoma	Gene rearrangement, integration and point mutations	1-2
NTRK	Adenocarcinoma	& integration Gene rearrangement	1-2
HER2	Adenocarcinoma	Introgression, point mutations and multiplication	1-5
PTEN	Adenocarcinoma	Copy number variant	1.7
PDGFRA	Adenocarcinoma	Copy number variant	6-7
PIK3CA	Adenocarcinoma	Copy number variant	5
TP53	Adenocarcinoma	Copy number variant	52
ERBB2	Adenocarcinoma	Copy number variant	2-5
TERT	Adenocarcinoma	Copy number variant	7.5
CDKN2A	Adenocarcinoma	Copy number variant	7
FGFR	Squamous cell carcinoma	Integration & Point mutation	23
TP53	Squamous cell carcinoma	Point mutation	79
NF1	Squamous cell carcinoma	Point mutation	10
DDR2	Squamous cell carcinoma	Point mutation	2-3
PDGFRA	Squamous cell carcinoma	Gene multiplication	4
PIK3CA	Squamous cell carcinoma	Gene multiplication	15
PTEN	Squamous cell carcinoma	Point mutation	10
SOX2	Squamous cell carcinoma	Copy number variant	8
CDKN2A	Squamous cell carcinoma	Gene multiplication & copy number variant	15

cancer in case of mutation. *RET* gene is a new oncogene located in chromosomal location 10q11.2 and has been reported in about 1.3% of lung cancer cases (42). Table 1 lists the names of oncogenes involved in the occurrence of lung cancer along with their frequency. Among the mutations in the genes involved in the occurrence of lung cancer, *EGFR*, *KRAS* and *BRAF* oncogenes along with the tumor suppressor gene *TP53* have the highest importance and role in the occurrence of lung cancer (42). Thus, identifying the mutations in all of this genes that most influence the development of lung cancer can be crucial to comprehending the pathophysiological mechanism of lung cancer. (40, 41).

EGFR gene mutations and the incidence of lung cancer

Epidermal growth factor receptor (EGFR) is a membrane glycoprotein that has tyrosine kinase activity (44) and is a member of the ErbB family. Other members of this family include ErbB2, ErbB3 and ErbB4 (47). EGFR plays an important role in regulating and controlling many different signaling pathways such as growth, cell proliferation, cell adhesion, differentiation, migration and survival (44-48). The epidermal growth factor receptor is coded by the *EGFR* gene, which is located at chromosomal location 7p12.1-12.3. This gene consists of 28 exons (44). EGFR activation as a result of epidermal growth factor binding leads to the activation of intracellular signaling cascades, which lead to the regulation and control of normal cell processes (47). The *EGFR* gene is the most important gene that plays a significant role in the risk of lung cancer at the genome level, in such a way that the presence of mutations in this gene leads to an increase in its expression and causes the conversion of the *EGFR* proto-oncogene into the *EGFR* oncogene, which as a result causes carcinogenesis in Different cells of the lung tissue. Therefore, by discovering the changes created, it is possible to predict and manage the process of carcinogenesis in the lung (20, 49, 50). According to Figure (1), binding of epidermal growth factor receptor to its ligand causes autophosphorylation through the activity of tyrosine kinase located in the second intracellular region and triggers several signal transmission cascades (51). *EGFR* signals activate at least two intracellular pathways in parallel. One of these pathways is the MAP kinase (MAPK) pathway, which regulates the G1 checkpoint in the cell cycle. When *EGFR* is activated, the MAPK pathway sends a signal to the nucleus through the active forms of *RAS*, *RAF* and *MEK* gene and causes cell proliferation (20). The presence of mutation on this gene increases its expression and leads to an increase in the signals sent to the nucleus as a result of the cell going out of normal reproduction and leading to the occurrence of cancer in different lung tissue cells (49). Mutations in the *EGFR* gene are often located on exons 18, 19, 20, and 21 (52). Among these, the most changes include deletions in exon number 19 and point mutation in exon number 21 of this gene (53). Two mutations, L858R and del exon 19, located on exons 21 and 19, respectively, have been reported in more than 90% of lung cancer

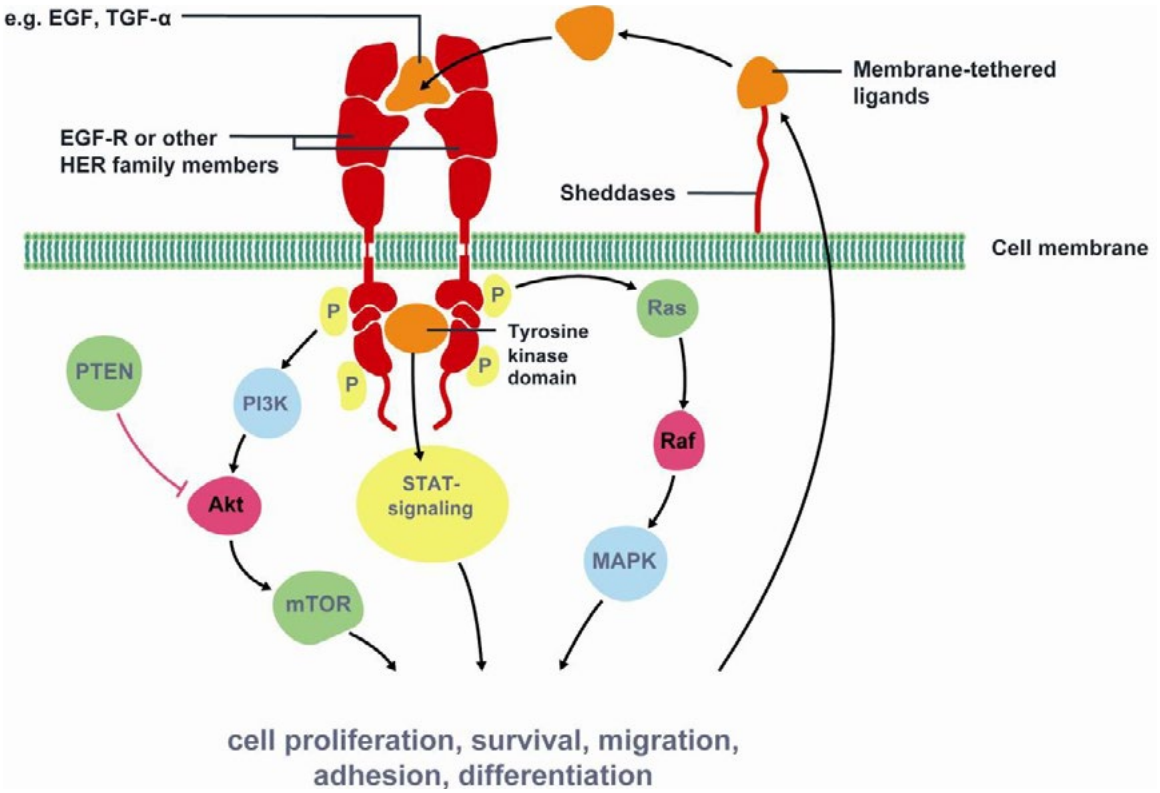

Fig 1. The role of the epidermal growth factor receptor (*EGFR*) gene pathway in the occurrence of lung cancer.

cases (54). The names of the mutations on exon 18 to 21 that play a role in the occurrence of lung cancer are given in Table 2.

These mutations in the *EGFR* gene are important from the point of view that they can be used in the field of diagnosis and treatment. Choosing a suitable treatment based on the molecular profile in the treatment of lung cancer in the field of personal medicine can play an important role in increasing the response to treatment, increasing the survival rate of patients, and reducing the mortality rate and material and spiritual damages. Identification and diagnosis of *EGFR* gene mutations and a correct understanding of the use of anti-*EGFR* tyrosine kinase inhibitors and the targeted use of common treatments (chemotherapy and radiation therapy) can play an important role in increasing the response rate to treatment, increasing patient survival, and reducing the death rate. and material and spiritual damages. Therefore, oncologists consider *EGFR* gene mutations to evaluate resistance to chemotherapy and radiation therapy and, if necessary, to use anti-*EGFR* tyrosine kinase inhibitors. The use of anti-*EGFR* tyrosine kinase inhibitors in lung cancer patients with *EGFR* mutations such as L858R and L861Q reduces the activity of tyrosine kinase and then prevents cancer progression (28, 55).

KRAS gene mutations and the incidence of lung cancer

KRAS gene, which is located at chromosomal position 12p12.1, is a member of the *RAS* family and one of the most important oncogenes involved in lung cancer. Activating mutations in the *KRAS* oncogene are the most common oncogenic changes in lung adenocarcinoma, which occur in up to 40% of cases (56, 57). According to Figure 2, KRAS is one of the G-proteins of the RAS family with GTPase activity, which is located in the inner space of the plasma membrane. Its function is that it acts as a double molecular switch. When it is bound to GTP, it is in the active state and when it is bound to GDP, it is in the inactive state. When it is active, the RAS-GTP complex activates several signaling cascades, such as the Raf-MEK-ERK, PI3K-AKT-mTOR and RalGDS-RalA/B pathways, as well as the TIAM1-RAC1 pathway. All these pathways play pivotal roles in cell proliferation, apoptosis, survival and growth (57). Activation of *KRAS* mutations prevents it from being hydrolyzed, so it remains permanently "on" and leads to continuous activity of downstream receptors (58). In other words, the mutation in the *KRAS* oncogene leads to a continuous message from the outside to the nucleus and then causes unregulated cell proliferation and the occurrence of cancer in lung tissue cells (59). Most mutations of this gene in cases of lung cancer occur on exon 2 and codons 12 and 13 of this gene (59, 60). These mutations are more than 95% in codon 12 and more than 80% in codon 13 of this gene. The most common mutated codon reported in lung cancer patients is the *KRAS -G12C* mutation, which has a frequency of about 40%. Other common mutations of this gene are G12V

Table2. Names of *EGFR* gene mutations involved in lung cancer

Mutation number	Exon	Nucleotide change	Amino acid change
1	18	c.2155G>A	p.G719S
2	18	c.2155G>T	p.G719C
3	18	c.2156G>A	p.G719D
4	18	c.2156G>C	p.G719A
5	19	c.2235_2249 del 15	Glu746_Ala750del
6	19	c.2235_2252> AAT	Glu746_Thr751delinsIle
7	19	c. 2236_2253 del 18	Glu746_Ser752del
8	19	c. 2237_2251 del 15	Glu746_Thr751delinsAla
9	19	c. 2237_2254 del 18	Glu746_Ser752>Ala
10	19	c. 2237_2255>T	Glu746_Ser752delinsVal
11	19	c. 2236_2250 del 15	Glu746_Ala750del
12	19	c.2238_2255 del 18	Glu746_Ser752delinsAsp
13	19	c. 2238_2248 >GC	Leu747_Ala750>Pro
14	19	c. 2238_2252 >GCA	Leu747_Thr751delinsGln
15	19	c. 2239_2247 del 9	Leu747_Glu749del
16	19	c. 2239_2253 del 15	Leu747_Thr751del
17	19	c. 2239_2256 del 18	Leu747_Ser752del
18	19	c. 2239_2258 >CA	Leu747_Pro753delinsGln
19	19	C. 2240_2251 del 12	Leu747_Thr751delinsSer
20	19	c. 2240_2257 del 18	Leu747_Pro753delinsSer
21	19	c. 2240_2254 del 15	Leu747_Thr751del
22	19	c. 2239_2251>C	Leu747_Thr751deinsPro
23	20	c.2369C>T	p.T790M
24	20	c.2303G>T	p.S768I
25	20	c.2307_2308insGCCAGCGTG	p.V769_D770insASV
26	20	c.2310_2311insGGT	p.D770_N771insG
27	20	c.2319_2320insCAC	p.H773_V774insH
28	21	c. 2573 T>G	Leu858Arg
29	21	c. 2582 T>A	Leu861Gln

and G12D with 21 and 18 percent respectively (61). The most important mutations of codons 12 and 13 of this gene are given in Table (3) (62). Identification of *KRAS* gene mutations can be used in the field of early diagnosis in the field of therapy using anti-*KRAS* tyrosine kinase inhibitors (28).

TP53 gene mutations and the occurrence of lung cancer

The most important tumor suppressor gene involved in the occurrence of lung cancer is TP53, which is located in the chromosomal position 17p13 and consists of 19,149 base pairs and 11 exons, which plays an important role in regulating the activity of genes involved in the processes of DNA repair, metabolism, cell cycle arrest, It has apoptosis and senescence, the result of which is the prevention of lung cancer. Mutations in this gene can cause lung cancer, as mutations in this gene have been reported in 50-60% of lung cancer cases (42, 45, 63). In healthy cells, P53 is normally present in a small amount in the cell. When

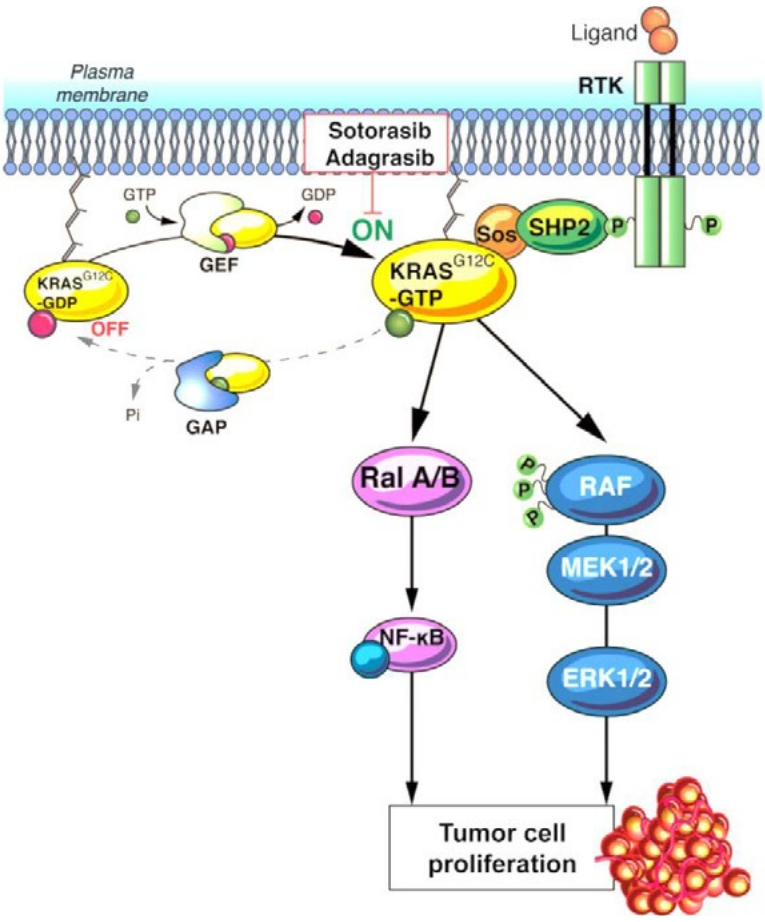

Fig 2. Schematic of molecular mechanism of mutation in *KRAS* gene and how lung cancer occurs .

cells are subjected to hypoxia stress or DNA damage, *P53* prevents its degradation and causes a rapid increase in its concentration inside the cell, so *P53* can bind to the promoter. DNA binds and stimulates the transcription of repair genes. If the damage caused cannot be repaired, then *P53* prevents the occurrence of cancer in lung tissue cells by transcription of genes involved in apoptosis and induction of apoptosis (63). Cells with mutated *P53* protein cannot bind to the DNA promoter sequence, so the damaged cell is not repaired and does not enter the path of apoptosis, and as a result, the path of cancer takes place (Figure 3). Such cells that lose their tumor suppressor gene activity are prone to multiply and become cancerous (63, 64).

More than 90% of the mutations that occur in this gene are located in the second DNA binding region of this gene. Most mutations of this gene in lung cancer are located on exons 5, 7, and 8 and codons 157, 158, 245, 248, and 273 (64, 63, 65). The most important mutations of this gene are given in Table (4). Knowing the mutations of this gene can also be beneficial in the diagnosis and treatment of patients. Several studies have shown that the presence of a mutation in the *P53* gene leads to resistance to lung cancer chemotherapy in laboratory and in vivo conditions. Knowing the status of *P53* is important and necessary to use chemotherapy and radiotherapy. Gene therapy is the best treatment solution in these cases (64).

BRAF gene mutations and the incidence of lung cancer

BRAF oncogene located at chromosomal position 7q34 is a member of the RAS family and mutations in this gene have been reported in up to 8% of lung cancer cases (42, 46). The *BRAF* gene is a serine/threonine kinase that controls cell proliferation by interacting with RAS-GTPs and downstream proteins from the MAPK family (28, 66). Mutation in exon 15 of this gene causes oncogenic activity in this gene and causes cancer in lung tissue cells (67). The most important and the only mutation reported in this gene in cases of lung cancer is V600E, which is located on exon number 15 of this gene. According to figure (4), The c.1799T>A (p.V600E) mutation of this gene causes the *BRAF* proto-oncogene to become the *BRAF* oncogene and the continuous activity of this gene, therefore, the natural growth and reproduction mode is out of reach and the cancerous process is started in different lung tissue cells. Therefore, the examination and testing of the *BRAF* gene mutation in lung cancer can be used as a diagnostic marker for early diagnosis. Also, this

Table 3. Names of *KRAS* gene mutations involved in lung cancer

Mutation number	Exon	Nucleotide change	Amino acid change
1	2	c.35G>A	G12D
2	2	c.34G>T	G12C
3	2	c.34G>A	G12S
4	2	c.34G>C	G12R
5	2	c.35G>C	G12A
6	2	c.35G>T	G12V
7	2	c.38G>A	G13D
8	2	c.37G > T	G13C
9	2	c.37G > A	G13S
10	2	c.37G > C	G13R
11	2	c. 175G>A	A59T
12	3	c. 181C>A	Q61K
13	3	c. 182A>T	Q61L
14	4	c. 182A>G	Q61R
15	4	c. 183A>C	Q61H
16	4	c. 183A>T	Q61H
17	4	c.351A>C	K117N
18	4	c. 351A>T	K117N
19	4	c. 436G>A	Al46T
20	4	c. 437C>T	A146V
21	4	c. 436G>C	A146P
22	4	c.35G>A	G12D

mutation can be used as a therapeutic target by using *BRAF* inhibitors to reduce cell proliferation and inhibit lung cancer progression (66- 68).

In this study, the genes involved in the incidence of lung cancer were identified and investigated. As a result of this study, it was determined that *EGFR*, *KRAS* and *BRAF* proto-oncogenes along with the *TP53* tumor suppressor gene showed the highest mutation frequency among all genes involved in lung cancer. The mentioned genes are known as central genes in lung cancer and have different hotspots for mutation. These hotspots in the *EGFR* gene are often located on exons 18, 19, 20 and 21, and among them, two mutations L858R and del exon 19 on exons 21 and 19, respectively, are included in more than 90% of lung cancer cases. In most of the studies conducted on the mentioned *EGFR* gene mutations, it was found that these mutations play a significant role in the field of response to treatment and tyrosine kinase inhibitors. The results of this study showed that the *KRAS* gene is the second most important proto-oncogene involved in the occurrence of lung cancer so most of the mutations of the mentioned gene in cases of lung cancer occur on codons 12 and 13 of exon 2 of this gene. These mutations are more than 95% in codon 12 and more than 80% in codon 13 of this gene. The most common mutation reported in patients is the *KRAS -G12C* mutation, which has a frequency of about 40%. Other common mutations of this gene are *G12V* and *G12D* with 21 and 18 percent respectively.

BRAF gene was identified as the third most important proto-oncogene involved in lung cancer. The

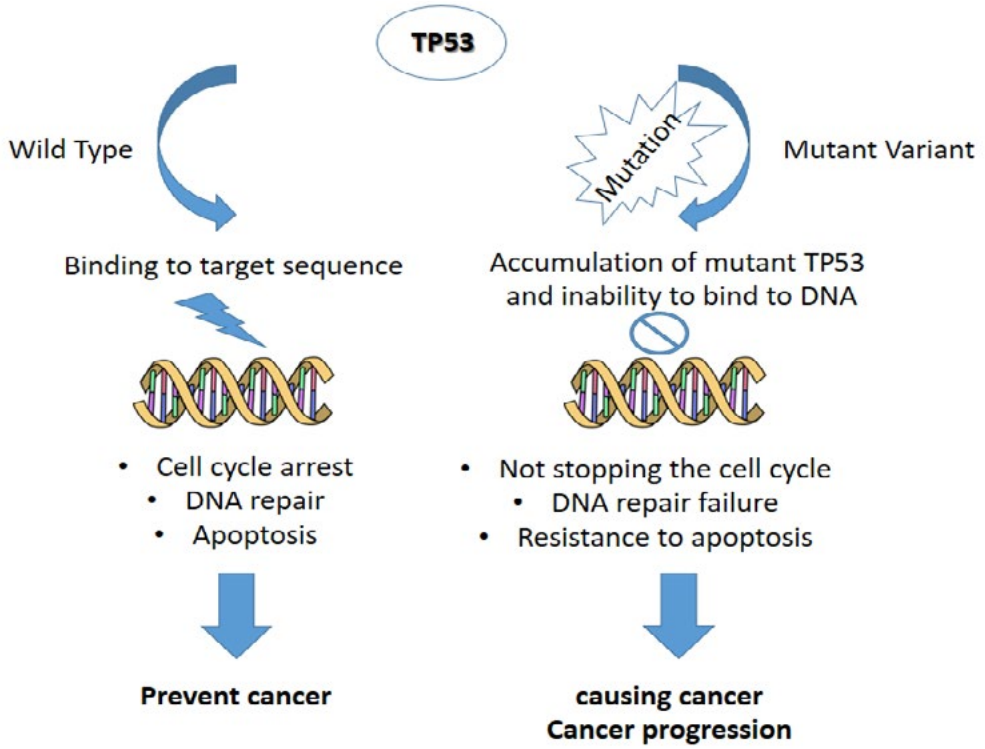

Fig 3. Schematic of how lung cancer occurs due to mutations in the *TP53* gene.

Table 4. Names of *TP53* gene mutations involved in lung cancer

Mutation number	Exon	Nucleotide change	Amino acid change
1	7	c.733G>T	p.G245C
2	7	c.741- 742CC>TT	p.R248W
3	8	c.818G>A	p.R273H
4	5	c.524G>T	p.R175L
5	7	c.743G>A	p.R248Q
6	7	c.747G>T	p.R249S
7	8	c.817C>T	p.R273C
8	5	c.430C>T	p.Q144*
9	5	c.438G>A	p.W146*
10	5	c.440T>A	p.V147D
11	5	c.472C>G	p.R158G
12	5	c.499C>T	p.Q167*
13	5	c.524G>T	p.R175L
14	7	c.722C>T	p.S241F
15	8	c.738G>C	p.M246I
16	8	c.818G>T	p.R273L

only mutation in this gene is c.1799T>A (p.V600E), which causes the BRAF proto-oncogene to become the *BRAF* oncogene, therefore, the natural growth and reproduction mode is out of reach and the cancerous

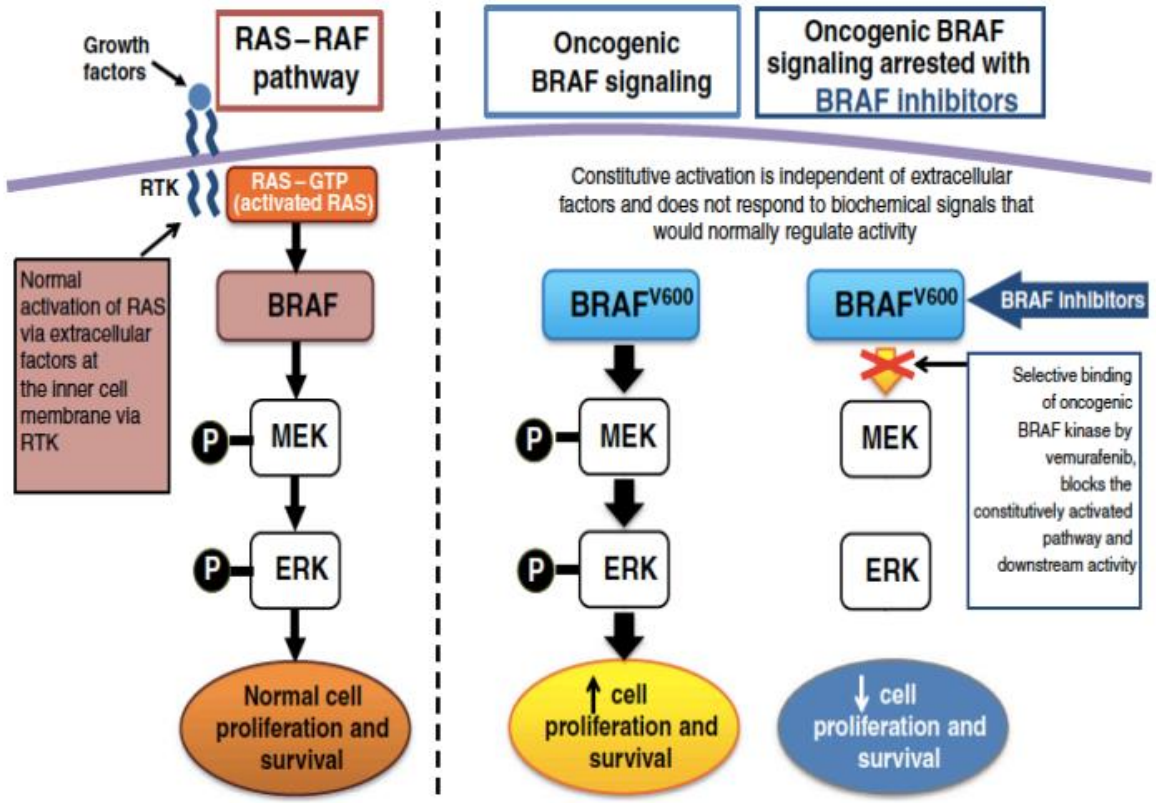

Fig4. Schematic of the molecular mechanism of the mutated *BRAF* gene and the incidence of lung cancer.

process is started in different lung tissue cells. The mentioned mutation test in the BRAF gene in lung cancer can be used as a diagnostic marker for early diagnosis and also as a therapeutic target by using BRAF inhibitors to reduce cell proliferation and inhibit the progression of lung cancer. The fourth gene and the only tumor suppressor gene that has the highest mutation rate in lung cancer is the *TP53* gene. The hotspots of mutation in this gene are exons 5, 7 and 8 and codons 157, 158, 245, 248 and 273. Knowing the mutations of this gene can also be beneficial in the diagnosis and treatment of patients in such a way that the presence of mutations in the *p53* gene leads to resistance to lung cancer chemotherapy in laboratory and in vivo conditions. Knowing the status of p53 is important and necessary to use chemotherapy and radiotherapy. Gene therapy is the best treatment solution in these cases. Examining the mutations of the mentioned genes, which have the highest mutation rate in the pathogenic process of lung cancer, can be used as clinical biomarkers for diagnosis, prognosis and response to treatment related to lung cancer.

DISCUSSION AND CONCLUSION

Several studies have been conducted on the frequency of mutations in the mentioned genes in people with lung cancer. In a study conducted by Li et al. in 2014 in China, the frequency of *EGFR* and *KRAS* gene mutations in lung tumors was studied. The mutation rate in the *EGFR* gene was reported to be 37% and in the *KRAS* gene to be 20%. Examining the mutation in these genes, in addition to predicting the risk of lung cancer, can be useful in the process of choosing a treatment strategy (52). In another study conducted by Wu et al. in 2012 in Taiwan, the status and frequency of *EGFR* gene mutations in patients with lung adenocarcinoma were analyzed using the sequencing method. In this study, about 70% of patients had mutations in the mentioned gene. The analysis of mutations of this gene can play an important role in early diagnosis and prognosis, as well as the selection of treatment methods related to the use of tyrosine kinase inhibitors (54). In a study conducted by Fouad et al. in 2012, the mutations of exon 4, 18, 19, 20 and 21 of the *EGFR* gene were investigated in lung tumor samples using the sequencing method. Mutations in the *EGFR* gene were observed in about 20% of tumor samples. Among the four exon mutations, the L858R mutation in exon 21 and deletion in exon 19 were the most frequent mutations (20).

In a study conducted by Fernando Lopez et al. 2012 in Spain, mutations in the *EGFR* gene were investigated in lung tumor samples. As a result of this study, more than 90% of mutation cases were reported, including deletions in exon 19 and point mutation L858R (77). In a study conducted by Yamamoto et al. 2017 in Japan,

the mutations of *EGFR* and *KRAS* genes in lung tumor samples were investigated using the NGS method. As a result of this study, mutations in *EGFR* gene were reported in about 40% of cases, and Ex19del and L858R mutations were the most frequent. Meanwhile, about 20% of mutation cases were observed in exon 2 of the *KRAS* gene (78). In the study conducted by Jing et al. in China, the mutations in *EGFR* and *TP53* genes were analyzed on paraffin-embedded tumor samples related to lung cancer by the NGS method, and as a result of this study, the mutation rate in the *EGFR* gene was equal to 52% and gene *TP53* was reported as 28% (79). In a study conducted by Tsiatis et al. in 2010, *KRAS* gene mutations in paraffin-embedded lung adenocarcinoma tumor samples were investigated with three methods, including sequencing, and as a result of this study, about 63% of the samples had mutations in the *KRAS* gene (59). In a study conducted by Grosse et al. in Switzerland on lung cancer patients, mutation frequency in *KRAS* oncogene was investigated using sequencing and NGS methods. As a result of this study, the rate of mutation in the *KRAS* gene was reported as 34% (19). In a study conducted by Jang et al. in 2009 in Korea, the mutations of *EGFR* and *KRAS* genes were evaluated using the sequencing method in patients with lung adenocarcinoma. As a result of this study, about 10 and 25% of mutations were reported in *KRAS* and *EGFR* genes, respectively (80). In a study conducted by Tuononen et al. in Finland, the mutations of *EGFR* and *KRAS* genes in paraffin samples of lung cancer tumors in the number of 81 samples were investigated using the NGS method. As a result of this study, about 25% of patients had mutations in EGFR and 33% had mutations in *KRAS* gene (81). Other researchers have investigated the mutation frequency of the *KRAS* gene in lung cancer patients, including Tsao et al. in 2010 about 34% mutation, Kern et al. in 1994 about 36% mutation, Capelletti et al. in 2010, 27 Regarding the percentage of mutation, Zhao et al. reported 22% mutation, Schmid et al. 37% mutation, and finally Schiller et al. 24% mutation in the *KRAS* gene in lung cancer patients (58, 65, 67). In a study conducted by Carter et al. in 2015 in Taiwan, the V600E mutation of the *BRAF* gene was investigated in lung, colon and melanoma cancer samples using the NGS method. As a result of this study, the mutation rate of this gene in lung cancer was reported as 6% (82). There have been many studies on the mutation frequency in the *TP53* gene. In the study conducted by Zhao et al. in 2019, *TP53* gene mutations were investigated in 50 patients with non-small cell lung cancer using sequencing techniques. As a result of this study, about 45% of mutations in the *TP53* gene were reported, and most of these mutations were located in exons 5, 7, and 8 of the *TP53* gene (65). In the study conducted by Shajani et al. in 2018, *TP53* gene mutations in colon, lung and glioblastoma cancers were investigated using the NGS method. As a result of this study, about 36% of people with lung cancer had mutations in *TP53* (78). In a study conducted by Labbe et al. in 2017, *TP53* gene mutations were investigated in lung tumor samples using NGS and sequencing techniques. As a result of this study, about 40% of the mentioned samples had mutations in the *TP53* gene (83).

In this study, genomic susceptibility regions that are related to the incidence of lung cancer were discussed and investigated. Further clinical-laboratory study of these areas related to lung cancer in different populations can be a beginning for designing a lung cancer screening panel to identify susceptible and high-risk individuals. The high lethality of lung cancer as well as diagnosis in advanced stages has forced researchers to discover and investigate molecular markers for the early diagnosis of this disease and also to evaluate the response to treatment.. Meanwhile, among the gene-offending regions, the most altered genes were those pertaining to the *EGFR, KRAS, TP53*, and *BRAF* genes. Consequently, studying the mutations of these genes in lung cancer can be crucial for clinical prediction, screening, early disease diagnosis, and treatment response assessment. Stated differently, the detection of these mutations as clinical biomarkers in the form of a screening panel can be crucial for early diagnosis, treatment process facilitation (by assessing treatment resistance), mortality reduction, and a decrease in the material and spiritual damages associated with this illness.

Declarations

Consent for publication

Not applicable.

Availability of data and material

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Funding

Not applicable.

Acknowledgment

The authors would like to express deep gratitude to the LifeandMe Company for giving the opportunity to conduct this study.

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