RESEARCH ARTICLE

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Mutational Damages in Malignant Lung Tumors

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Abstract

Background: Today, genomic changes are an important cause of the occurrence, growth and progression of cancer. Technological advances in cancer genomic analysis platforms have made it possible to identify genomic alterations that may influence response to lung cancer treatment. **Methods:** The study examined tumor growth-inhibiting oncogenes and genes responsible for cell growth and division to identify mutations characteristic of malignant lung tumors. The mutations were studied in 400 postoperative samples after amplifying p53 and HRAS fragments and p53, p21Waf1, MDM2 mRNA. p53 or p21Waf1 were expressed in 50% of squamous cell carcinomas and adenocarcinomas of the lung. **Results:** The study examined tumor growth-inhibiting oncogenes and genes responsible for cell growth and division to identify mutations characteristic of malignant lung tumors. The mutations were studied in 400 postoperative samples after amplifying p53 and HRAS fragments and p53, p21Waf1, MDM2 mRNA. p53 or p21Waf1 were expressed in 50% of squamous cell carcinomas and adenocarcinomas of the lung. HRAS mutations were present in most squamous cell carcinomas and adenocarcinomas of the lung. EcoR1- and Pst1- restriction enzymes destroyed the RT-PCR product of the p53 and p21Waf1 mRNA and increased the level of detected mutations in lung adenocarcinoma to 75% and 50 %, respectively. EGFR mutations were more frequent in lung adenocarcinoma than in lung squamous cell carcinoma. Mutations in EGFR exons 19 and 21 found in 65 of 263 lung tumor samples indicated the tumor sensitivity to EGFR tyrosine kinase inhibitors. EGFR deletions in exon 19 occurred mainly in adenocarcinoma, L858R mutations in EGFR exon 21 were quite common in lung adenocarcinoma. **Conclusion:** The mutations detected in most squamous cell carcinomas and adenocarcinomas of the lung could be used to diagnose and predict the disease severity and targeted therapy efficacy.

Keywords: Gene mutations- lung- adenocarcinoma- carcinoma- cell

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Introduction

Many genes, signaling pathways, and chromosome regions are already associated with lung cancer (Singhal, 2005). Abnormal expression or dysfunction of tumor suppressor genes was found in tumor protein P53 (p53), multiple tumor suppressor 1 (p16), Retinoblastoma protein (Rb), Fragile Histidine Triad Diadenosine Triphosphatase (FHIT), Ras association (RalGDS/AF-6) domain family member 1 (RASSFF1A), Semaphorin 3B (SEMA3B), phosphatase and tensin homolog deleted on chromosome 10 (PTEN), 8-Oxoguanine glycosylase (hOGG1), and Big Anchor Cell (BAC1) (Ghosh et al., 2022; Turganbekova et al., 2017; Tatenov et al., 2015).

Genomic alterations are an important cause of cancer initiation, growth, and progression. Various technological advancements in cancer genomic analysis platforms made it possible to identify genomic alterations that may affect the response to treatment for lung cancer. Therefore, genetics/genomics research is actively conducted in the field of lung cancer. Such factors as epidermal growth factor receptor (EGFR), Anaplastic lymphoma kinase (ALK), and proto-oncogene 1 (ROS1) were identified and are currently used as targets in lung cancer treatment. Data regarding mutations in such genes as Serine/threonine-protein kinase B-raf (BRAF), Vascular endothelial growth factor (VEGF), Kirsten rat sarcoma virus (KRAS), rearranged during transfection (RET), and N-methyl-N'-nitroso-guanidine human osteosarcoma transforming gene (MET) is used for cancer treatment in addition to immunological markers such as programmed cell death (PD)-1 and PD ligand 1 (PD-L1) (El-Telbany, 2012).

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The adenocarcinomas with no EGFR, KRAS, or ALK mutations (the so-called triple-negative adenocarcinoma) had much higher expression of baculoviral inhibitor of apoptosis repeat-containing 5 (BIRC5), Minichromosome Maintenance Complex Component 4 (MCM4), cell division cycle protein 20 homolog (CDC20), PCNA-associated factor (KIAA0101), and Thyroid Hormone Receptor Interactor 13 (TRIP13) (Cao, 2019).

Mutations in the TP53 tumor-suppressor gene are common in human cancers. TP53 encodes p53, and the mutations in TP53 inactivate the normal activity of p53. However, some mutations increase the cancer-promoting p53 gain-of-function (GOF). It is still unknown whether various p53 mutants produce similar GOF-related activities and how they do so. Finding the answer could support the development of strategies for treating many cancers (Prives, 2015). Chromosome deletions, truncations, and missense mutations in genes encoding TP53 and RB are the most common changes found in 90% of small cell lung carcinomas (Prives, 2015; Muller, 2013). Numerous biological studies have revealed increased activity of the epidermal growth factor receptor (EGFR) and the cascade it triggers in patients with non-small cell lung carcinoma (NSCLC) (Omar et al., 2022; George, 2015).

Modern treatment of lung cancer is type- and stage-dependent. Tissue biopsy is made to determine the type of cancer and potential molecular characteristics (Paez, 2004). Detecting a genetic mutation and finding an agent active against this change allows targeting the tumor. EGFR is the best example of genetic mutations found in lung cancer, for which biological agents were subsequently developed (George, 2015). Changes in EGFR were found in approximately 10% of NSCLC patients. Treatment of such patients with tyrosine kinase inhibitors (TKIs), i.e., gefitinib or erlotinib, proved clinically useful. Other single-gene mutations found in lung cancer include liver kinase B1 gene (LKB19), echinoderm microtubule-associated protein-like 4 and anaplastic lymphoma kinase (EML4-ALK), KRAS, human epidermal growth factor receptor 2 (Her2), and BRAF (Lindeman, 2013; Hiroyuki, 2005).

The aim of the work is to study mutations of tumor growth suppressor genes such as p53, cyclin-dependent kinase inhibitor 1A wild-type p53-activated fragment ($p21^{WAF1}$) and Mouse double minute 2 homolog (MDM2), as well as the genes involved in cell division control, such as EGFR and transforming protein p21 (HRAS GTPase), which control one of the main ways of cell growth gene activation and might be used to predict the severity of the disease and the effectiveness of targeted therapy.

Materials and Methods

The object of research was 200 (n=5 series x 40 samples) samples of squamous cell carcinoma and adenocarcinoma of the lung and 200 (n=5 series x 40 samples) samples of adjacent healthy tissue taken from the postoperative material, and 263 samples of various lung tumors taken from freshly frozen tissue or tissue fixed with formalin and enclosed in paraffin. Deoxyribonucleic Acid (DNA) was extracted using Bioline ISOLATE II Genomic DNA Kits. Exons-introns 5-6 and 7-9 of the p53 gene and sections of the *HRAS* gene containing codons 12 and 61 were amplified by polymerase chain reaction (PCR) in the presence of primers specific to those sections and selected using the "PRIMER" program.

The reverse transcriptase-polymerase chain reaction (RT-PCR) was performed using the AmpliSens REVERTA-L-100 reagent kit according to the manufacturer's instructions. For that purpose, 10 μL of the extracted Ribonucleic acid (RNA) was added to 10 μL of the reaction mixture containing hexanucleotide primers, RNA eluent, deoxynucleotide triphosphates, ribonuclease inhibitor, and 200 U of reverse transcriptase (EC2.7.7.49). The obtained mixture was incubated for 30 min at 37°C. The synthesized first complementary DNA (cDNA) strand was then introduced into the mixture to copy (amplify) DNA fragments in PCR.

PCR was carried out using Hybaid Omn-E Thermal Cycler as follows: one cycle at 95°C for 5 min, 30 subsequent cycles at 94°C for 20 s, 55-56°C for 20 s and 72°C for 40 s, concluded by one cycle at 72°C for 5 min. The reaction mixture (25 μL) contained 30 ng of each primer, 0.5 μM of each deoxynucleotide triphosphate, 5 μL of DNA or cDNA, 2.5 U of Tag-polymerase (EC2.7.7.7) and 5 μL of 10-fold PCR buffer. PCR products were detected by ethidium bromide incorporation after electrophoresis in 1.5-2% agarose gels in ultraviolett (UV) transilluminator.

At the second stage, site-specific endonucleolysis of PCR products of DNA and cDNA fragments was conducted using EcoR1 and Pst1-restriction enzymes (EC 3.1.21.4). The restriction was performed in the incubation medium containing 5 μL of PCR product, 1.25 μL of H–buffer from the restriction enzyme kit, one unit of restriction enzyme in 1 μl. The total sample volume was brought to 12.5 μL by water. The samples were incubated at 37°C for 1 h, then cooled in ice and dispersed in 2% agarose gel electrophoresis. The electrophoregrams were dyed with ethidium bromide and analyzed by the luminous intensity in UV using the "Gel analysis" software.

Real-time PCR was carried out to evaluate the EGFR gene mutations in 263 lung cancer samples. Tumor DNA was isolated from fresh frozen tissue or formalin-fixed and paraffin-embedded tissue. Real-time PCR to detect L858R mutation in exon 21 and deletions in exon 19 of the EGFR gene was performed following the instructions to the Real-time-PCR-EGFR-2M" reagent kit. The kit contained three PCR mixtures: one control mix, one mix for L858R mutations, and one mix for deletions in exon 19. The EGFR gene PCR products were identified in a 5'-nuclease reaction using a FAM-labeled probe. The PCR mixtures also contained ROX. Reference Dye to detect PCR inhibitors able to deliver false-negative results.

Microsoft Excel was used to process the experimental results, determine the Pearson correlation coefficient, calculate the Student's t-criterion and the significance level P. The explanation of the calculation method is available as online supplementary material.

Results

Mutational Damages in Adenocarcinoma of the Lung

In adenocarcinoma of the lung, the p53 gene fragments containing exons-introns 5-6 and 7-9 were found in all studied cancerous and adjacent tissues (n = 5 series x 20 samples). $P21^{WAF1}$ messenger RNA (mRNA) and p53 mRNA were silent in 50% of cancerous and 25% of adjacent tissues. MDM2 mRNA was expressed in 75% of cancerous and only 25% of adjacent tissues. The criterion $t > 2$, $r_{xy} > 0.8$ corresponded to the probability of error-free forecast of $p > 95.5\%$. Those differences across all mRNA-containing samples indicated cell polymorphism in both cancerous and adjacent tissues. Degradation of p53 exon-intron 5-6 fragments caused by restriction enzyme treatment and $p21^{WAF1}$ decrease caused by MDM2 mRNA expression were more frequent in adenocarcinoma samples than in adjacent tissues (Tables 1, 2).

After restriction enzyme treatment, the number of adenocarcinoma adjacent tissues containing PCR products of p53 mRNA, HRAS12, and p21WAF1 mRNA decreased by 50%. However, the enzyme treatment did not affect the number of adenocarcinoma and adjacent tissue samples containing mRNA MDM2 and HRAS61 products. mRNA MDM2 was found in 75% of adenocarcinoma and 25% of adjacent tissues; HRAS61 – in 50% of adenocarcinoma and 60% of adjacent tissues (Table 2).

Mutational Damages in Squamous Cell Carcinoma of the Lung

PCR product of the p53 gene with exon-intron 5-6 and 7-9 was much less often in squamous cell carcinoma samples than in adjacent tissues (Table 3). The $p21^{WAF1}$ and p53 mRNA PCR products were absent in 50% of lung squamous cell carcinoma and a significant part of adjacent tissues. MDM2 mRNA PCR product was found in 16-17 out of every 20 samples of squamous cell carcinoma and 12 out of 20 adjacent tissue samples (Table 3).

The restriction enzyme treatment reduced the number of squamous cell carcinoma samples containing p53 PCR products with exons-introns 5-6 and 7-9 and the number of adjacent tissue samples containing p53 exon-intron PCR products. The treatment of squamous cell carcinoma samples with EcoR1- and Pst1 destroyed the p53 and *MDM2* mRNA and HRAS12 PCR products and reduced the number of adjacent tissue samples containing such products. However, the treatment did not affect the number of squamous cell carcinoma and adjacent tissue samples

Table 1. The Presence of p53 PCR Products and mRNA of Cell Proliferation Control Genes in Lung Adenocarcinoma (X) and Adjacent Tissue (Y) Samples

Series of samples[a]	p53 exon-intron 5-6		WAF1 mRNA product		MDM2 mRNA		p53 mRNA		p53 exon-intron 7-9	
	X	Y	X	Y	X	Y	X	Y	X	Y
1	20	20	14	19	19	9	15	19	20	20
2	20	20	11	17	17	7	11	14	20	20
3	20	20	8	12	12	4	6	12	20	20
4	20	20	10	13	11	2	8	16	20	20
5	20	20	7	14	16	3	10	14	20	20
M_{av}[1]	$M_x=\sum x/n$	$M_y=\sum y/n$	$M_x=\sum x/n$	$M_y=\sum y/n$	$M_x=\sum x/n$	$M_y=\sum y/n$	$M_x=\sum x/n$	$M_y=\sum y/n$	$M_x=\sum x/n$	$M_y=\sum y/n$
	20	20	10	15	15	5	10	15	20	20
r_{xy}[d]	No differences		0.845402793		0.834440867		0.808053408		No differences	
t[d]	No differences		2.741434646		2.622574067		2.37577257		No differences	

Note: [a], Each series contained 20 samples; [b],The t-criterion > 2 and $r_{xy} > 0.8$ correspond to the probability of an error-free forecast of differences between the compared values of $p > 95.5\%$.

Table 2. The Presence of p53, HRAS or $p21^{WAF1}$ PCR Product, MDM2 Gene Fragment or mRNA in Lung Adenocarcinoma and Adjacent Tissues before and after Restriction

	Lung adenocarcinoma		Enzyme treated samples of lung adenocarcinoma		Lung adenocarcinoma adjacent tissue		Enzyme treated samples of lung adenocarcinoma adjacent tissue		Proportion of mutations in enzyme-treated samples	
Gene fragment, mRNA	No. of samples	% of the series[a]	No. of samples	% of the series	No. of samples	% of the series [b]	No. of samples	% of the series [b]	Among lung adenocarcinoma samples, %	Among samples of lung adenocarcinoma adjacent tissue, %
p53 (exon-intron 5-6)	20	100	10.0±5.0	50	20	100	15.0±4.32	75	50 (-50%)*	75 (-25%)
p53 (exon-intron 7-9)	20	100	20	100	20	100	20	100	100 (0)	100 (0)
p53 mRNA	10.0±5.0	50	5.0±3.54	25	15.0±4.32	75	10.0±4.08	50	25 (-75%)	50 (-50%)
HRAS codon 12	15.0±4.32	75	5.0±4.08	25	15.0±4.32	75	10.0±4.08	50	25 (-75%)	50 (50%)
HRAS codon 61	10.0±5.0	50	10.0±5.0	50	12.0±4.38	60	12.0±4.38	60	50 (-50%)	60 (-40%)
p21WAF1 mRNA	10.0±5.0	50	5.0±3.54	25	15.0±4.32	75	10.0±4.08	50	25 (-75%)	50 (-50%)
MDM2 mRNA	15.0±4.32	75	15.0±4.32	75	5.0±4.32	25	5.0±4.32	25	75 (-25%)	25 (-75%)

*, (% mutations); [a], Each series contained 20 samples

Table 3. The Presence of p53 PCR Products and mRNA of Cell Proliferation Control Genes in Lung Squamous Cell Carcinoma (X) and Adjacent Tissue (Y) Samples

Series of samples[a]	p53 exon-intron 5-6		pWAF1 mRNA product		MDM2 mRNA		p53 mRNA		p53 exon-intron 7-9	
	X	Y	X	Y	X	Y	X	Y	X	Y
1	15	19	14	7	19.7	16.4	15	9	16	20
2	8	17	11	4	17.4	15	13	8	13	19.75
3	9	15	8	5	13.5	10	8	4	8	19.2
4	11	16	10	3	14	6.6	11	5.6	13	20
5	14	19	7	1	18.7	12	10	2	10	19.8
M_{av}[b]	$M_x=\sum x/n$	$M_y=\sum y/n$	$M_x=\sum x/n$	$M_y=\sum y/n$	$M_x=\sum x/n$	$M_y=\sum y/n$	$M_x=\sum x/n$	My=∑y/n	Mx=∑x/n	My=∑y/n
	11.4	17.2	10	4	16.66	12	11.4	5.72	12	19.75
r_{xy}[d]	0.806559133		0.775671752		0.8306044		0.858641109		0.828740916	
t[d]	2.363179084		2.128704008		2.583488549		2.901435841		2.564935856	

Note: [a], Each series contained 20 samples; [b],The t-criterion > 2 and r_{xy} > 0.8 correspond to the probability of an error-free forecast of differences between the compared values of p > 95.5%.

containing HRAS61 and mRNA p21WAF1 PCR products, which was already low (Tables 4). Such silencing of tumor growth suppressor genes and the *MDM2* gene in adjacent tissue indicated a malfunction in cell growth and division control (Table 4).

At that, mutational damages were found in 100% of squamous cell carcinoma samples containing the p53 mRNA, MDM2 mRNA, and HRAS12 PCR products, as well as in 50-85% of squamous cell carcinoma samples containing other PCR products. No mutations were found in squamous cell carcinoma adjacent tissues containing p53 PCR product with exon-intron 7-9. PCR products of other gene fragments and other mRNAs were not detected in 43-86% of samples of normal tissues adjacent to squamous cell carcinoma.

Mutations in EGFR

24.7% (65 of 263) of cancerous samples had deletions in EGFR exon 19 and mutations in EGFR exon 21. 18.5% (32 of 173) of samples of metastatic lung adenocarcinomas with different localization in the lungs had deletions in exon 19. Eight percent (14) of samples had missense mutations with leucine to arginine replacement in exon 21, L858R, sensitive to EGFR inhibitors (Table 5). Three out of 34 lung squamous cell carcinoma samples had deletions in EGFR exon 19, with no L858R replacement in EGFR exon 21. Six samples of bronchioloalveolar cancer had a deletion in exon 19, and one sample had a mutation in exon 21. In one mixed cancer sample, the deletion was detected in exon 19. The deletions in exon 19 were found in single lung adenocarcinoma metastases, NSCLC, and small cell lung carcinoma samples. Leucine to arginine

Table 4. The Presence of PCR Product of p53, HRAS, $p21^{WAF1}$, or MDM2 Gene Fragment or mRNA in Lung Squamous Cell Carcinoma and Adjacent Tissues before and after Restriction

*Gene fragment, mRNA	Lung squamous cell carcinoma		Enzyme treated samples of lung squamous cell carcinoma		Lung squamous cell carcinoma adjacent tissue		Enzyme treated samples of lung squamous cell carcinoma adjacent tissue		Proportion of mutations in enzyme-treated samples*	
	No. of samples	% of the series[a]	No. of samples	% of the series	No. of samples	% of the series	No. of samples	% of the series[b]	Among lung squamous cell carcinoma samples, %	Among samples of lung squamous cell carcinoma adjacent tissue, %
p53 (exon-intron 5-6)	11.4±3.71	57	5.7±2.84	28.5	17.14	86	11.44±3.30	57.2	28.5 (-70%)	57.2 (-50%)
p53 (exon-intron 7-9)	12.0±4.38	60	8.0±3.26	40	20	100	20	100	40 (-60%)	100 (0)
p53 mRNA	11.4±4.38	57	0	0	5.7±3.42	28.5	2.86±2.02	14.3	0 (-100)	14.3 (-85,7%)
HRAS codon 12	14.3±3.42	71.5	0	0	14.2±3.40	71.4	11.32±2.46	56.6	0 (-100)	56.6 (-43,4%)
HRAS codon 61	3..0±2.70	15	3.0±2.7	15	6.66±3.80	33.3	6.66±3.84	33.3	15 (-85%)	33.3 (-66,7%)
p21WAF1 mRNA	10.0±4.80	50	10.0±4.08	50	4.0±3.58	20	4..0±3.58	20	50 (-50%)	20 (-80%)
MDM2 mRNA	16.7±3.04	83.5	0	0	12.0±4.38	60	8.00±2.84	40	0 (-100)	40 (-60%)

*, (% mutations); [a], Each series contained 20 samples

Table 5. EGFR Mutations (del 19 exon and L858R 21 exon) in Different Histological Forms of Lung Cancer

No. of mutations	Lung squamous cell carcinoma	Lung adenocarcinoma	Undifferentiated lung carcinoma	Adenocarcinoma metastases	NSCLC	SCLC	Bronchioloalveolar cancer	Mixed cancer
not found	31	127	3	30	3	0	0	4
del 19 exon	3	32	3	1	1	1	6	1
L858R	0	14	1	0	0	1	1	

replacement, L858R, in EGFR exon 21 was detected in single samples of undifferentiated cancer, small cell lung carcinoma, and bronchioloalveolar cancer.

In our study, 75% of lung cancer samples had no mutations of interest. That did not preclude the presence of other deletions, missense-mutations, or increased copy number of the EGFR gene, as well as the mutations in the RAS family, p53, $p21^{WAF1}$, MDM2, and other genes that control various intracellular signal transmission pathways and can stimulate cell reproduction or block these signals.

Discussion

TP53 (which encodes p53 protein) is another most frequently mutated gene in all human cancers. Prevalent p53 missense mutations abrogate its tumor-suppressive function and lead to cancer-promoting GOF. Zhu et al., (2015) showed that p53 GOF mutants bind to and upregulate chromatin regulatory genes, including the methyltransferases MLL1 (also known as KMT2A), MLL2 (also known as KMT2D), and acetyltransferase MOZ (also known as KAT6A, or MYST3). All this widely increases genome methylation and histone acetylation. The Cancer Genome Atlas analysis shows specific MLL1, MLL2, and MOZ activation in p53 GOF patient-derived tumors, but not in wild-type p53 or p53-null tumors (Zhu, 2015). Cancer cell proliferation is markedly lowered by genetic knockdown of MLL1 or by pharmacological inhibition of the MLL1 methyltransferase complex. Zhu et al., (2015) revealed a novel chromatin mechanism underlying the progression of tumors with GOF p53. They suggested new possibilities for designing combinatorial chromatin-based therapies for treating individual cancers driven by prevalent p53 GOF mutations.

In our study, lung squamous cell carcinoma was associated with p53 gene damage in exons-introns 5-6 and 7-9 and lung adenocarcinoma – with p53 gene damage in exon-intron 5-6. No mutations were found in p53 exon-intron 7-9 in lung adenocarcinoma and the tissues adjacent to lung squamous cell carcinoma or lung adenocarcinoma. Despite this, $p21^{WAF1}$ and p53 silencing were found in 50% of lung adenocarcinoma and lung squamous cell carcinoma and 70-80% of adjacent tissues. MDM2 mRNA was expressed in 75-85% of lung adenocarcinoma and lung squamous cell carcinoma samples and only in 25% and 60% adjacent tissues. MDM2 mRNA expression suppresses the transactivating activity of the p53 protein towards the p53, $p21^{WAF1}$, and MDM2 genes (Tables 2, 4).

The detected damage to the p53 gene and the lack of p53 and $p21^{WAF1}$ expression in 50% of lung adenocarcinoma and lung squamous cell carcinoma samples violated the cell growth, differentiation, and apoptosis rates.

Recent studies by several authors (Ding, 2018; Sui, 2019; Meliala, 2020; Cao; 2015) showed that the transactivation of p21 (cyclin-dependent kinase inhibitor 1A) is closely related to the recruitment of transcription cofactors at the p53 responsive elements (p53REs) in its promoter region. Human chromatin remodeling enzyme INO80 can be recruited to the p53REs of the p21 promoter and negatively regulates p21 (Ding, 2018). Interestingly, high expression of p21 was observed in most morphologically changed cells, suggesting that negative regulation of p21 by the INO80 complex might be implicated in maintaining the cell cycle process and chromosome stability.

The protein-protein interaction between p53 and its negative regulator MDM2 comprises one of the most important and intensely studied protein-protein interactions involved in cancer prevention. This interaction is based on conformation and is tightly regulated at several levels. The molecules with a Co-crystalline structure were developed that inhibit p53-MDM2/X interaction (Khoury, 2021). Inhibiting this interaction of wild-type p53 has become an important target in oncology to restore the antitumor activity of p53, which is called the guardian of our genome. Interestingly, based on the multiple disclosed compound classes and structural analysis of small-molecule-MDM2 adducts, the p53-MDM2 complex is perhaps the best-studied and most targeted protein-protein interaction. Several classes of small molecules were identified as potent, selective, and efficient inhibitors of the p53-MDM2/X interaction, and many co-crystal structures with this protein were identified (Estrada-Ortiz, 2016).

In our study, restriction enzymes destroyed the RT-PCR product of p53 and $p21^{WAF1}$ mRNA, increasing the level of detected mutations in lung adenocarcinoma to 75%. Restriction enzymes did not destroy the RT-PCR product of MDM2 mRNA in lung adenocarcinoma and adjacent tissues (Table 2). EcoR1- и Pst1- restriction enzymes destroyed the PCR products of p53 and MDM2 mRNA in all lung squamous cell carcinoma samples and a part of adjacent tissue samples, which indicated the appearance of sequences recognized by those restriction enzymes (Table 4). Possible differences in the mutation spectrum in lung squamous cell carcinoma, lung adenocarcinoma and adjacent tissue could determinate these tumors' growth and metastasis. Thus, the mutations in the DNA-binding domain in p53 exon-intron 5-6 suppress the transactivation function in both cancerous and adjacent tissues regardless of the histological type of tumor. This dysregulates the p53-dependent genes' expression, such $asp21^{WAF1}$, and MDM2.

Ras oncogenes (Hras, Kras, Nras) are important drivers of carcinogenesis. However, tumors with Ras mutations often show a loss of the corresponding wild-type allele, suggesting that proto-oncogenic forms of Ras can suppress carcinogenesis. Deletion of one Hras allele dramatically reduces the number of skins papillomas in Hras mutations; therefore, Hras is the major target of mutation in these tumors. Still, skin carcinoma indices are very similar, so wild-type Hras probably functions as a suppressor of papillomas' progression into invasive squamous carcinomas (To et al., 2013).

Ras gene mutations are observed in more than 30% of all cancers. They are more prevalent in some difficult-to-treat malignancies, such as more than 90% of pancreatic cancers, as well as lung and colon cancers. Ras proteins (N-Ras, H-Ras, K-Ras) act as molecular switches. When activated, they bind GTP to initiate a cascade of signaling events that control important cellular processes such as proliferation and division. RAS gene mutations were

identified in codons 12, 13, 59, and 61, while codon 12 and 61 mutations are most frequent. In NSCLC, the prominent mutation is detected in position one of the K-ras gene codon 12 (wild-type nucleotide sequence GGT). Guanine (G) is predominantly replaced by thymine (T) (G-T transversion) what represents a 'hot spot' of ras point mutation. In lung cancer, H-ras or N-ras mutations are found only in exceptional cases. In general, ras mutations are 15-60% more frequent in lung adenocarcinoma than lung squamous cell carcinoma. K-ras mutations are also found in most large cell carcinomas (Asfar, 2017).

The mutations in both HRAS12, p53, and MDM2 mRNA were found in 100% of lung squamous cell carcinoma samples and 43.4%, 85.7%, and 60% adjacent tissue samples, respectively (Table 4). The mutations in both HRAS12, p53 mRNA, and $p21^{WAF1}$ mRNA were found in 75% of lung adenocarcinoma and 50% of adjacent tissues (Table 2). HRAS61 mutations were present in 50% of lung adenocarcinoma and 40% of adjacent tissue, 85% of lung squamous cell carcinoma and 66.7% of adjacent tissues ((Table 4). Such a positive correlation between the HRAS12 and p53 mRNA mutations in cancerous and adjacent tissues indicated the interaction between the mutant p53 and HRAS12 products (Table 2). HRAS12 mutations were found in 75% of lung squamous cell carcinoma samples vs. 25% after restriction. No sequences specific to those restriction enzymes were found in HRAS61 fragments in lung squamous cell carcinoma, lung adenocarcinoma, or adjacent tissue (Tables 2, 4).

KRAS is the most frequently mutated gene in human cancer. Despite its direct involvement in malignancy and intensive effort to study it, direct KRAS inhibition by pharmacological inhibitors is still challenging. RNAi-induced knockdown using siRNAs against mutant KRAS alleles offers a promising tool for selective therapeutic silencing in KRAS-mutant lung cancers. However, the major bottleneck for clinical translation is the lack of efficient biocompatible siRNA carrier systems. BSA nanoparticles loaded with mutant-specific siRNA are a promising therapeutic approach for KRAS-mutant cancers. Nanoparticle uptake, the cellular distribution of nucleic acids, cytotoxicity, and gene knockdown that can interfere with cancer hallmarks, uncontrolled proliferation, and migration were evaluated in KRAS G12S A459 mutant cells, a lung adenocarcinoma cell line (Mehta, 2019).

Lung cancer is the most painful and fatal disease among other cancers. There are several standard therapies for lung cancer, such as surgery, immunotherapy, radiotherapy, and chemotherapy. It urges the need for more targeted and efficient methods of treating lung cancer. E.g., RNAi, in combination with traditional therapy, could silence genes involved in drug resistance. Such genes can motivate the inhibition of apoptosis, promote the epithelial-mesenchymal transition and DNA repair. These genes can participate in intracellular signaling pathways such as JAK/STAT, RAS/RAF/MEK, PI3K/AKT, NICD, B-catenin/TCF/LEF. Stimulant receptors, including IGFR, EGFR, FGFR, VEGFR, CXCR4, MET, INTEGRINS, NOTCH1, and FRIZZLED, can also be considered as suitable targets (Naghizadeh, 2019). In lung adenocarcinoma, the targetable major pathways include EGFR, PI3K/AKT/mTOR, RAS-MAPK, and NTRK/ROS1 pathways (Singh, 2020; Liu, 2017). Many drugs targeting these pathways have been developed and shown clinical benefits (Kawano, 2006). Nevertheless, while target therapy in NSCLC has provided disease control, the tumors inevitably develop drug resistance.

Thus, the differences in lung adenocarcinoma and lung squamous cell carcinoma mutations could determine these tumors' histological and morphological features. Knowing these differences could help develop adequate diagnostic and treatment approaches in lung adenocarcinoma and lung squamous cell carcinoma.

The mutations in EGFR exons 19 and 21 were detected in 65 (24.7%) out of 263 lung cancer samples studied (Table 5). These mutations increase the sensitivity to EGFR TKIs, which is why nearly 25% of lung tumors are sensitive to EGFR TKIs (i.e., gefitinib, erlotinib) (Da Cunha Santos, 2011; Dahabreh, 2010). A lack of positive effect of targeted therapy is most likely due to the p53 exon-intron 5-6 and HRAS12 mutations. The resistance mechanisms should be understood, and combinational therapies developed to improve the treatment outcomes (Ma, 2019). The known mechanisms of drug resistance in NSCLC include TK domain mutation (T790M), MET amplification, RAS mutation (Fumarola, 2014; Sukauichai et al., 2022; Pérez-Ramírez, 2015; Wang, 2018).

Several genetic alterations that aberrantly activate tyrosine kinases were identified as oncogenic drivers in NSCLC, and many TKIs were developed to target such drivers (Takeuchi, 2012; Sordella, 2004; Shaw, 2013). Among them, chromosomal rearrangements of anaplastic lymphoma kinase (ALK), ROS proto-oncogene 1 (ROS1), rearranged during transfection (RET) and neurotrophic receptor tyrosine kinases (NTRKs) are each observed in 0.1-5% of NSCLC patients (Takeuchi, 2012; Shaw, 2013; Rikova, 2007). ROS1 gene rearrangements are observed in about 1-2% of NSCLC cases, as well as in cholangiocarcinoma, glioblastoma, ovarian, gastric, and colorectal cancers (Lin, 2017).

Advances in targeted therapy medications over the past decade suppress lung cancer progression while improving patient survival and quality of life (Yuan, 2019; Martin, 2020). Such drugs as Tagrisso (osimertinib), Tarceva (erlotinib), and Iressa (gefitinib) act as TKIs since they prevent the EGFR protein on mutated cells from triggering tyrosine kinase, which turns on cell division and thus multiplies cancer cells (Zappa, 2016). Tagrisso is now recommended as the first-line therapy for EGFR mutations due to its better ability to penetrate the cerebrospinal fluid and pass through the blood-brain barrier to help fight lung cancer with brain metastases. It is important because lung cancer often spreads to the brain (Reddi, 2013). While the EGFR gene is most often associated with lung adenocarcinoma, some lung squamous cell carcinoma types are also affected by the EGFR protein. In these cancers, growth is not related to a mutation but to EGFR amplification, which causes rapid growth and results in highly aggressive tumors. To treat these lung cancer cells, the doctors use a monoclonal antibody (human-made antibody) such as Portrazza (necitumumab) that blocks

the activity of EGFR (Liam, 2019).

In conclusions, the detected damage to the p53 gene and the lack of p53 and p21^{WAF1} expression in 50% of lung adenocarcinoma and lung squamous cell carcinoma samples violated the cell growth, differentiation, and apoptosis rates. Possible differences in the mutation spectrum in lung squamous cell carcinoma, lung adenocarcinoma and adjacent tissue could determinate these tumors' growth and metastasis. Thus, the mutations in the DNA-binding domain in p53 exon-intron 5-6 suppress the transactivation function in both cancerous and adjacent tissues regardless of the histological type of tumor. This dysregulates the p53-dependent genes' expression, such asp21^{WAF1}, and MDM2. Such a positive correlation between the HRAS12 and p53 mRNA mutations in cancerous and adjacent tissues indicated the interaction between the mutant p53 and HRAS12 products. Thus, the differences in lung adenocarcinoma and lung squamous cell carcinoma mutations could determine these tumors' histological and morphological features. Knowing these differences could help develop adequate diagnostic and treatment approaches in lung adenocarcinoma and lung squamous cell carcinoma. A lack of positive effect of targeted therapy is most likely due to the p53 exon-intron 5-6 and HRAS12 mutations. The resistance mechanisms should be understood, and combinational therapies developed to improve the treatment outcomes. Advances in targeted therapy medications over the past decade halt lung cancer progression while improving patient survival and quality of life.

The *p53* gene mutations in exon-intron 5-6 were detected in 70% of lung squamous cell carcinoma and 50% of lung adenocarcinoma samples, in exon-intron 7-9 – in 60% of lung squamous cell carcinoma samples. The p53 gene functioning was disrupted by the truncated and mutant mRNAs, found in 100% of lung squamous cell carcinoma and 85% of lung squamous cell carcinoma adjacent tissues, 75% of lung adenocarcinoma and 50% of lung adenocarcinoma adjacent tissues. EcoR1- and Pst1- restriction enzymes destroyed the RT-PCR product of the p53 and p21^{WAF1} mRNA and increased the level of detected mutations in lung adenocarcinoma to 75% and 50%, respectively. The destruction of the p53 and MDM2 mRNA PCR products by the EcoR1- and Pst1-restriction enzymes in all lung squamous cell carcinoma and a part of adjacent tissues indicated the presence of sequences recognized by those restriction enzymes. The restriction enzymes did not destroy the RT-PCR MDM2 mRNA product in lung adenocarcinoma and adjacent tissues. In general, EGFR mutations were more frequent in lung adenocarcinoma than in lung squamous cell carcinoma. EGFR deletions in exon 19 occurred mainly in adenocarcinoma.

Author Contribution Statement

SY, MO: Conceptualization, Data curation, Investigation, Project administration, Resources, Software, Supervision, Writing – original draft, Writing – review & editing. TG, FR, ZD, TV: Formal Analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Ethics approval and consent to participate

This observational study followed the ethical standards of the Helsinki Declaration of 1964 as amended and the requirements of the local ethics committee of Kazakh Institute of Oncology and Radiology (No. LEK-II/2008-01-007 in the Register of ethics committees of Kazakhstan).

Supplementary Material

Supplementary material can be found at http://carcin.oxfordjournals.org/

Data availability statement

The data underlying this article are available in the article and in its online supplementary material.

Conflict of interests

The authors declare that they have no conflict of interest.

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