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Araştırma Makalesi / Research Article

Akciğer Skuamöz Hücreli Karsinomda Apolipoprotein B Gen Mutasyonu ve Promotor Metilasyon Durumunun Biyoinformatik Değerlendirilmesi

Bioinformatic Evaluation of Apolipoprotein B Gene Mutation and Promotor Methylation Status in Lung Squamous Cell Carcinoma

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Öz

Amaç: Literatürde apolipoproteinler ile kanser mortalitesi arasındaki bağlantıya dair yeterli kanıt bulunmamaktadır. Bu nedenle bu çalışmada Akciğer Skuamöz Hücreli Karsinomu (LUSC) ile ApoB genindeki mutasyonlar arasındaki olası ilişkinin araştırılması amaçlandı.

Gereç ve Yöntem: cBioPortal aracılığıyla TCGA veri tabanından toplam 469 kanser LUSC hastası dahil edildi. Genel sağkalımı (OS) değerlendirmek için Kaplan Meier Plott veritabanı kullanıldı. Promotör metilasyon seviyesi UALCAN veri tabanı tarafından belirlendi. Gen ekspresyon düzeyi GEPIA2 ile araştırıldı.

Bulgular: LUSC kohortunda ApoB geninde toplam 83 mutasyon tespit edildi. LUSC örneklerinde ApoB geni ekspresyon düzeyleri sağlıklı örneklere göre düşüktü ancak bu istatistiksel olarak anlamlı değildi ($p>0.05$). LUSC kohortunda ApoB geninin ne yüksek ne de düşük ekspresyon seviyeleri OS ile ilişkili değildi. ApoB geninin promotör bölgesindeki hipometilasyon istatistiksel olarak anlamlıydı ($p<0.05$)

Sonuç: ApoB genindeki değişiklikler ile LUSC arasındaki ilişki tam olarak açık değildir. Bu nedenle ApoB'nin anti-kanser tedavilerinde potansiyel bir hedef veya biyobelirteç olarak değerlendirilmesinin prospektif çalışmalarla desteklenmesi gerektiğini düşünüyoruz.

Anahtar Kelimeler: Apolipoprotein B, Akciğer kanseri, Gen mutasyonu

Abstract

Aim: There is insufficient evidence in the literature about the link between apolipoproteins and cancer mortality. For this reason, the current study aimed to investigate the possible relationship between Lung Squamous Cell Carcinoma (LUSC) and mutations in the ApoB gene.

Material and Method: A total of 469 patients with cancer LUSC were included in the TCGA database via cBioPortal. The Kaplan Meier Plott database was used to evaluate overall survival (OS). The promoter methylation level was determined by the UALCAN database. The gene expression level was investigated by GEPIA2.

Results: A total of 83 mutations in the ApoB gene were detected in the LUSC cohort. ApoB expression levels were decreased in LUSC samples compared to healthy samples, but this was not statistically significant ($p>0.05$). Neither high nor low expression levels of the ApoB gene were associated with OS in the LUSC cohort. Hypomethylation in the promoter region of the ApoB gene was statistically significant ($p<0.05$)

Conclusion: The relationship between alterations in the ApoB gene and LUSC needs to be clarified. Therefore, prospective studies should support the evaluation of ApoB as a potential target or biomarker for anti-cancer treatments.

Keywords: Apolipoprotein B, Lung cancer, Gene mutation

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INTRODUCTION

The incidence of lung cancer is increasing globally and remains the leading cause of cancer deaths. Lung cancer is an intricate and diverse condition distinguished by the irregular growth and varying rates of multiplication of cells in the lungs. There are various risk factors, but smoking is the predominant risk factor[1,2]. Lung cancer presents diversity; the majority of lung cancers are non-small cell lung cancers, including squamous cell carcinoma, adenocarcinoma, and large cell carcinomas. Among these, adenocarcinoma stands out as the most prevalent form of lung cancer[3,4]. ApoB serves a crucial function in the transport of lipoproteins and acts as the primary regulatory protein for many of them. While research on ApoB predominantly concentrates on cardiometabolic disorders, its connection to cancer remains less understood. However, heightened serum levels of ApoB have been linked to conditions like diabetes and metabolic syndrome, both of which have the potential to influence cancer development[5].

Furthermore, there are studies suggesting that elevated levels of ApoB are associated with an increased risk of lung and colorectal cancer. At the same time, lower concentrations of ApoB are linked to a heightened risk of breast cancer[6]. In contrast, studies are showing that a low ApoA/ApoB ratio, i.e., high ApoB levels, is associated with an increased risk of lung and colorectal cancer[7].

Studies investigating the association between ApoB and cancer mortality have controversial results. For this reason, we investigated the relationship between possible mutations and promotör methylation in the Apo B gene and Lung Squamous Cell Carcinoma (LUSC) by using some bioinformatics tools in this study.

MATERIAL AND METHODS

Working group design

This is a bioinformatic study. The present research utilized openly available data from The Cancer Genome Atlas (TCGA), a public repository accessible at https://www.cancer.gov/tcga. Ethical clearance has been obtained from the relevant authorities to include patients' data within this database. Researchers can freely access and download pertinent data for scientific investigation and subsequent publication of findings. No ethical concerns or conflicting interests are associated with using this data. The LUSC dataset comprising 469 samples was acquired from the cbioPortal database, which offers open-access bioinformatics tools and data sourced from TCGA. The data retrieval occurred on August 10, 2023.

Evaluation of mutation

The mutation profile analysis of the ApoB gene in patients with LUSC was conducted using the cBioPortal web tool. This analysis utilized the Oncoprint interface feature provided by the tool. Additionally, various aspects, including amino acid position and localization, nucleotide change, cancer subtype, cancer stage, histological grade, and co-expression levels of selected genes, were evaluated using the cBioPortal web tool. Furthermore, the COSMIC database was consulted to confirm whether somatic mutations were present.

Survival analysis

The KM Plot (https://kmplot.com/analysis/) is an online resource offering gene expression and clinical data for analyzing the correlation between gene expressions and cancer survival rates[8]. This tool investigated the prognostic significance of ApoB gene expression levels in patients diagnosed with LUSC. Furthermore, the analysis encompassed the examination of overall survival (OS) outcomes among LUSC patients based on the expressions of associated genes.

Gene expression analysis

GEPIA2 (http://gepia2.cancer-pku.cn/#index) is an enhanced version of GEPIA, providing a web-based platform for comparing tumor tissues with normal tissues. In addition to existing features such as differential expression analyses, spectrogram plotting, correlation analyses, and patient survival analyses, GEPIA2 introduces new functionalities, including survival maps, isoform usage profiling, comparisons of uploaded expression data, and cancer subtype classifiers. It also allows users to customize analyses by uploading their RNA-seq data and comparing it with samples from TCGA and GTEx while enhancing some of the original functionalities[9].

Methylation analysis

UALCAN is an interactive open-access web page for OMICS data analysis (http://ualcan.Path.uab.edu/index.html). This database is built on PERL-CGI and can be used at approximately 6000 gene methylation levels[10]. This study evaluated the promoter methylation level of the ApoB gene in the LUSC. Promoter region methylation levels of the ApoB gene were also examined in LUSC subtypes.

Statistical analysis

Data from the TCGA Pan-Cancer Atlas, obtained via cBioPortal, was utilized to gather patient information and statistically assess the mutation types within the ApoB gene. Additionally, statistical analyses were conducted using the GEPIA2 database, employing the one-way ANOVA test to evaluate differential expression. Survival analysis was performed using the KM plotter, with default settings, focusing on recurrence-free survival (RFS) and utilizing auto-best cutoff values and the J-best probe set. The optimal threshold for cutoff values, encompassing all possible values between the lower and upper quartiles, was determined, and the most suitable threshold was selected for analysis. A log-rank p-value of less than 0.05 was considered statistically significant.

RESULTS

Mutation profile

The cBioPortal web tool was used to analyze changes in ApoB protein in LUSC patients. Among 469 cases, 83 (17,7%) of LUSC patients had genetic changes in ApoB. The types of mutations encountered in the ApoB gene in LUSC are shown in Table 1. A missense mutation (73 mutations, 88%) was the most common type of change encountered in mutations in genes, while one splice (1.2%), one FS deletion (1.2%), and eight nonsense (9.6%) were found. The number of male and female patients with mutations was 63 (76%) and 20 (24%), respectively. Of the mutations analyzed, none were identified as originating from germ cells, except those classified as unknown mutations. All mutations, except those of unknown origin, were confirmed to be somatic. In cases where missense mutations were observed in the examined genes, they led to phenotypic alterations in the protein. The nature of these changes varied depending on the type of missense mutation, whether conservative or nonconservative. Such mutations alter the mRNA's codon, encoding a different amino acid due to a change in base pairs. The functional consequences of these mutations may vary, potentially impairing or preserving the protein's function. Important mutations in the 100% conserved domains of the ApoB gene in the evolutionary process include the p. X302_splice mutation in the Vitellogenin_N domain, the p. S964* nonsense mutation in the DUF1081 domain, and the p. E4511* nonsense mutation in the Apolipoprotein B100 C-terminal domain. Information on amino acid changes and the effects of all other mutations are shown in Table 1.

Survival analysis

The GEPIA2 (http://gepia2.cancer-pku.cn/#index) analysis assessed 5-year overall survival (OS). Data on the OS assessment are shown in Figure 1. When the OS results were evaluated, increased expression levels of the ApoB gene were found to be statistically associated with shorter OS (p=0.045).

Analyzes of promoter methylation level

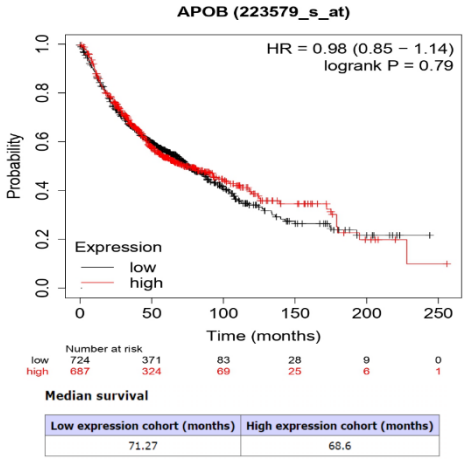
DNA methylation plays a crucial role in modifying the genome epigenetically and is intricately linked to the cancer developmental process[11]. UALCAN is a comprehensive, user-friendly, and interactive web resource for analyzing cancer OMICS data. It is built on PERL-CGI with high-quality graphics using JavaScript and CSS. UALCAN is designed 10. According to the results of the analysis using the UALCAN online tool to determine the DNA methylation level, the promoter methylation level of ApoB in LUSC tissues was lower (hypomethylation) compared to healthy tissues (p=0.024). However, this was not statistically significant. The results of this are shown in Figure 2.

Table 1: Demographic, clinical, and genetic data of patients with LUSC

No	Nucleotide Change	Amino Acid Position	Variation Type	Gender	Diagnosis Age	Domain	Lymph Node Stage	Tumor Stage	Metastasis Stage Code	Neoplasm Disease Stage
M-1	c.13564G>T	D4522Y	MS	Male	81	ApoB100_C	N1	T2	M0	STAGE IIB
M-2	c.13531G>T	E4511*	NS	Male	72	ApoB100_C	N0	T2	M0	STAGE IB
M-3	c.13348G>A	E4450K	MS	Male	69	NDR	N0	T3	M0	STAGE IIB
M-4	c.12598C>A	P4200T	MS	Male	72	NDR	N0	T2	M0	STAGE IB
M-5	c.12577C>G	L4193V	MS	Female	67	NDR	N0	T1A	M0	STAGE IA
M-6	c.12575C>A	S4192*	NS	Female	66	NDR	N0	T2A	MX	STAGE IB
M-7	c.12547C>A	H4183N	MS	Female	73	NDR	N0	T2	MX	STAGE IB
M-8	c.12325C>A	Q4109K	MS	Female	66	NDR	N1	T2	M0	STAGE IIB
M-9	c.12262T>A	Y4088N	MS	Male	70	NDR	N0	T3	M0	STAGE IIB
M-10	c.12261G>T	K4087N	MS	Male	44	NDR	N1	T2A	M0	STAGE IIA
M-11	c.11966G>A	R3989H	MS	Male	61	NDR	N0	T2	M0	STAGE IB
M-12	c.11675C>T	P3892L	MS	Male	71	NDR	N0	T1B	MX	STAGE IA
M-13	c.11507C>T	A3836V	MS	Male	74	NDR	N0	T4	MX	STAGE IIIB
M-14	c.11365G>A	V3789I	MS	Female	60	NDR	N0	T2	M0	STAGE IB
M-15	c.11162G>A	E3788K	MS	Female	73	NDR	N0	T2	NA	STAGE IB
M-16	c.11339C>A	A3780D	MS	Male	63	NDR	N0	T2	M0	STAGE IB
M-17	c.11239C>G	P3747A	MS	Male	72	NDR	N0	T1	M0	STAGE IA
M-18	c.10988G>A	G3663E	MS	Male	73	NDR	N0	T1	MX	STAGE IA
M-19	c.10906G>A	E3636K	MS	Male	54	NDR	N0	T2A	MX	STAGE IB
M-20	c.10554C>A	N3518K	MS	Male	79	NDR	N0	T3	M0	STAGE IIB
M-21	c.10445G>A	S3482N	MS	Male	60	NDR	N0	T2A	MX	STAGE IB
M-22	c.10061C>A	A3354D	MS	Male	57	NDR	N0	T2B	M0	STAGE IIA
M-23	c.9907G>T	E3303*	NS	Male	65	NDR	N2	T2	M0	STAGE IIIA
M-24	c.9873G>A	R3291H	MS	Male	70	NDR	N0	T2A	MX	STAGE IB
M-25	c.9794C>A	A3265D	MS	Male	65	NDR	N1	T4	NA	STAGE IIIB
M-26	c.9682del	I3228Lfs*38	FS del	Male	69	NDR	N0	T2	M0	STAGE IB
M-27	c.9612T>A	F3204L	MS	Female	73	NDR	N0	T2A	M0	STAGE IB
M-28	c.9583G>C	E3195Q	MS	Female	65	NDR	N2	T2A	M0	STAGE IIIA
M-29	c.9266G>T	W3087L	MS	Male	64	NDR	N2	T2	M0	STAGE IIIA
M-30	c.9194C>T	T3065I	MS	Male	70	NDR	N0	T2A	MX	STAGE IB
M-31	c.8840G>C	S2947T	MS	Male	70	NDR	N0	T2A	M0	STAGE IB
M-32	c.8143G>T	E2715*	NS	Male	64	NDR	N0	T2	M0	STAGE IB
M-33	c.8057G>A	W2686*	NS	Male	70	NDR	N1	T2	M1	STAGE IV
M-34	c.7815C>G	L2605V	MS	Female	76	NDR	N0	T1	M0	STAGE IA
M-35	c.7639C>T	V2547I	MS	Male	57	NDR	N0	T2B	M0	STAGE IIA
M-36	c.7558C>T	R2520*	NS	Female	70	NDR	N0	T1	M0	STAGE IA
M-37	c.7075G>C	D2359H	MS	Male	68	NDR	N0	T2A	M0	STAGE IB
M-38	c.7054C>T	Q2352*	NS	Male	69	NDR	N0	T2A	MX	STAGE IB
M-39	c.6994G>A	E2332K	MS	Male	58	NDR	N1	T3	M0	STAGE IIIA
M-40	c.6994G>T	E2332D	MS	Male	83	NDR	N0	T2A	M0	STAGE IB
M-41	c.6910A>G	T2304A	MS	Male	71	NDR	N0	T2	M0	STAGE IB
M-42	c.6863A>T	H2288L	MS	Male	83	NDR	N1	T2	M0	STAGE IIB
M-43	c.6731G>T	S2244I	MS	Male	66	NDR	N2	T2	M0	STAGE IIIA
M-44	c.6271G>T	V2091L	MS	Male	65	NDR	N0	T2	M0	STAGE IB
M-45	c.6223C>G	E2075Q	MS	Female	62	NDR	N1	T3	M0	STAGE IIIA
M-46	c.6146C>G	P2049R	MS	Male	70	NDR	N0	T3	M0	STAGE IIB
M-47	c.6145C>G	P2049A	MS	Male	70	NDR	N0	T3	M0	STAGE IIB
M-48	c.5774G>T	G1925V	MS	Male	59	NDR	N0	T3	M0	STAGE IIB
M-49	c.5425A>T	T1809S	MS	Male	59	NDR	N2	T2	M0	STAGE IIIA
M-50	c.5410A>G	N1804D	MS	Male	57	NDR	N0	T4	M0	STAGE IIB
M-51	c.5305A>G	K1769E	MS	Male	73	NDR	N0	T4	M0	STAGE IIIA
M-52	c.4961C>A	T1654N	MS	Male	71	NDR	N0	T2A	M0	STAGE IB
M-53	c.4952G>T	G1651V	MS	Female	60	NDR	N0	T3	M0	STAGE IIB
M-54	c.4710A>C	L1570F	MS	Male	60	NDR	N0	T3	M0	STAGE IIB
M-55	c.4399G>C	A1467P	MS	Male	76	NDR	N1	T4	M0	STAGE IIIB
M-56	c.4384G>A	G1462R	MS	Male	68	NDR	N0	T2	M0	STAGE IB
M-57	c.4382G>T	W1461L	MS	Female	74	NDR	N0	T2A	MX	STAGE IB
M-58	c.4193G>T	D1398Y	MS	Male	63	NDR	N0	T1B	M0	STAGE IA
M-59	c.4143G>C	D1381H	MS	Male	74	NDR	N0	T3	M0	STAGE IIB
M-60	c.3943G>T	E1314D	MS	Male	68	NDR	N0	T1	M0	STAGE IA
M-61	c.3676G>T	V1226L	MS	Male	60	NDR	N1	T2	M0	STAGE IIB
M-62	c.3675C>A	H1225Q	MS	Male	78	NDR	N0	T2	M0	STAGE IB
M-63	c.3323G>A	G1108D	MS	Female	76	NDR	N0	T1	M0	STAGE IA
M-64	c.3051C>A	S1017R	MS	Male	70	DUF1081	N0	T3	M0	STAGE IIB
M-65	c.2991C>A	S997*	NS	Male	67	DUF1081	N1	T1A	MX	STAGE IIA
M-66	c.2410C>T	R804C	MS	Female	69	DUF1943	N1	T2	M0	STAGE IIB
M-67	c.2265G>C	M755I	MS	Male	69	DUF1943	N0	T2	M0	STAGE IB
M-68	c.2249G>T	D749Y	MS	Male	69	DUF1943	N0	T2A	M1B	STAGE IV
M-69	c.2202A>T	L734F	MS	Male	83	DUF1943	N0	T1	MX	STAGE IA
M-70	c.2170G>T	G724C	MS	Female	70	DUF1943	N0	T1B	M0	STAGE IA
M-71	c.1960G>T	G657W	MS	Female	45	DUF1943	N0	T3	M0	STAGE IIB
M-72	c.1903C>T	R635W	MS	Male	78	NDR	N0	T3	M0	NA
M-73	c.1792G>T	A598S	MS	Female	47	NDR	N1	T3	M0	STAGE IIIA
M-74	c.1715C>A	A572E	MS	Female	56	Vitellogenin_N	N1	T2B	M0	STAGE IIB
M-75	c.1668T>A	D556E	MS	Male	79	Vitellogenin_N	N0	T3	M0	STAGE IIB
M-76	c.1428G>C	D477H	MS	Male	55	Vitellogenin_N	N0	T4	M0	STAGE IIIB
M-77	c.1318G>T	A440S	MS	Female	59	Vitellogenin_N	N0	T1	M0	STAGE IA
M-78	c.1213C>T	P405S	MS	Male	67	Vitellogenin_N	N0	T3	M0	STAGE IIB
M-79	c.1133C>T	T378I	MS	Male	64	Vitellogenin_N	N2	T2	M0	STAGE IIIA
M-80	c.904-1A>G	X302_splice	Splice	Male	83	Vitellogenin_N	N0	T2A	M0	STAGE IB
M-81	c.877A>G	K293E	MS	Male	63	Vitellogenin_N	N0	T2	MX	STAGE I
M-82	c.563C>T	T188I	MS	Male	56	Vitellogenin_N	N0	T1B	M0	STAGE IA
M-83	c.301G>C	E101Q	MS	Male	74	Vitellogenin_N	N1	T3	M0	STAGE IIIA

Gene expression profiling interactive analysis results

According to the results of the comparison of expression levels in LUSC patients (n=486) and healthy participants (n=338) via ApoB (http://gepia2.cancer-pku.cn/#index) database,



Median survival

Low expression cohort (months)	High expression cohort (months)
71.27	68.6

Figure 1: Different expressions of the ApoB gene in LUSC patients in the overall survival (OS) curve (using the Kaplan-Meier plotter). The red line represents the survival rate curve of patients with LUSC who expressed the gene, and the black line represents the survival rate curve of LUSC patients who did not express the gene.

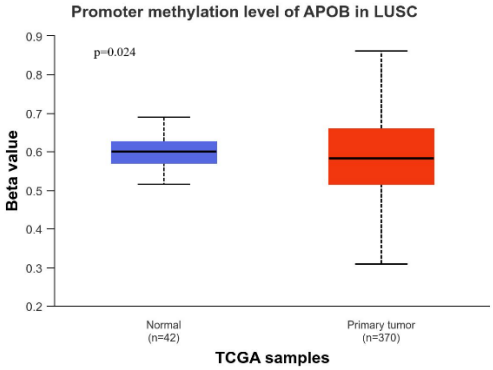


Figure 2: The Promoter Methylation Level of ApoB gene in LUSC

no statistically significant distinction was observed in the expression levels of the examined genes between tumor tissue and normal tissue (p>0.05). In addition, expression levels according to LUSC subtypes (basal, classical, primitive, and secretory) and staging were not statistically different compared to healthy participants (Figure-3) (p>0.05).

DISCUSSION

In this study, 83 mutations were detected in 469 LUSC samples. Of these, 8 (9.7%) were nonsense mutations, 1 (1.2%) was splice mutation, 1 (1.2%) was frameshift deletion, and 73 (87.9%) were missense mutations. To the best of our knowledge, our study is the first to use bioinformatics tools to explore the association between the ApoB gene and LUSC.

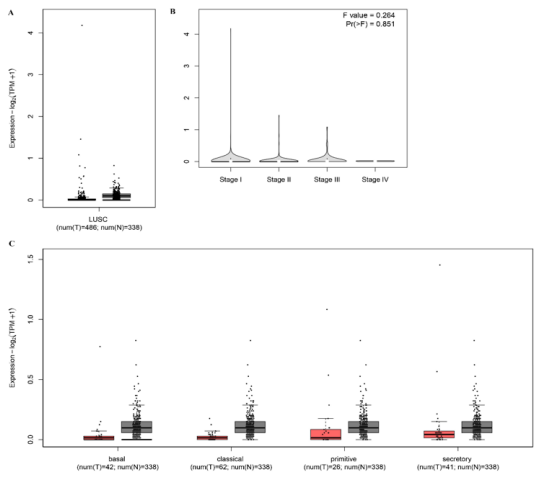


Figure 3: A- mRNA expressions of ApoB in LUSC (red) and normal breast tissues (gray). B- The expression level of the ApoB gene in LUSC is based on stages (p=0.851). C- The expression level of ApoB gene in LUSC based on major subclasses (basal, classical, primitive, secretory) * p<0.05

Apolipoproteins (Apo's) bind to lipids to form lipoproteins. Lipids are critical in creating the structural basis of biological membranes and signaling molecules[12]. Apart from this, it is thought that Apo's are potential biomarkers in the diagnosis and prognosis of many malignancies such as lung cancer, gastric cancer, and colorectal cancer and that Apo's have a role in tumorigenesis and cancer progression[13]. Increasing evidence in recent years has shown that Apo's participate in classical cancer pathways involving PI3K/Akt, MAPK, and Wnt signaling[13]. The PI3K-Akt signaling pathway is a multifaceted regulatory network in human malignant tumors. It plays a pivotal role in governing tumor progression by regulating various aspects such as cell proliferation, genomic stability, and metabolism[14]. One of the genes with which the ApoB gene interacts in our LUSC cohort was the FGR gene, a member of the Src family of protein tyrosine kinases. The FGR gene is a protooncogene and increases the phosphorylation of phosphatidylinositol 3-kinase regulatory subunit (PIK3R1)[15]. This can show efficiency in PI3K/Akt regulation.

ApoB is located on chromosome 2p24.1 and encodes ApoB, the major apolipoprotein of chylomicrons and low-density lipoprotein (LDL)[16]. While LDL is known as the "bad cholesterol" for both heart and vascular disease, the functional role of cholesterol and its transporter, ApoB, in cancer growth remains unclear. Increased cellular cholesterol levels can increase the proliferation and migration of cancer cells, possibly leading to tumor progression[17]. While studies argue that ApoB has severe effects on LDL and that increased lipid levels are associated with cancer deaths, including lung cancer, they say that ApoB may be a potential adjuvant method for future lung cancer treatments[18]. In this case, mutations that occur in the 100% conserved splice regions of the ApoB gene and affect the formation of truncated protein may affect lipid metabolism by creating severe effects on the protein function[19]. It is stated that truncated changes in the ApoB gene cause familial hypobetalipoproteinemia (FHBL), and these mutations increase continuously in different subjects[20]. In addition, it is indicated that inactivating mutations in the ApoB gene disrupt VLDL particle metabolism, and triglyceride levels increase 3 times in cancer patients compared to healthy individuals[20]. Lipid metabolism disorders play a vital role in the pathogenesis of squamous cell carcinoma. It is argued that disorders in lipid metabolism in lung squamous cell carcinoma may help understand the biological behavior of the tumor[21]. In the LUSC cohort, 9 mutations can cause the formation of truncated proteins.

The most important is the p. E4511* nonsense mutation in the ApoB100 C terminal domain. Nonsense mutation in this domain can result in the formation of a short and nonfunctional polypeptide chain. ApoB100 is essential in the assembly and secretion of triglyceride-rich lipoproteins and lipids transport[22]. In this case, in addition to defective lipid transport, it is possible to experience problems in the secretion of triglyceride-rich lipoproteins. Apart from this mutation, mutations in the gene or its regulatory region can cause diseases that affect ApoB levels, ligand-defective ApoB-induced hypercholesterolemia, normotriglyceridemic hypobetalipoproteinemia, and hypobetalipoproteinemia[23].

In addition, in cases where mutations can form this truncated protein, there are mutations known as recurrent hotspots (statistically significant) in different genes. The p.S964 nonsense mutation mainly accompanies the X51_splice change in the CDKN2A gene. The CDKN2A gene encodes two proteins, p16INK4A and p14ARF, which regulate cell growth and survival. Such a mutation occurring

in this gene may lead to decreased binding to CDK4 and CDK6, resulting in an inability to inhibit the cell cycle[24,25]. In addition, p.V155F missense mutation in the KEAP1 gene occurred in the case with p.E4511* nonsense mutation in the ApoB100 C terminal region. This change is a recurrent hotspot located in the KEAP1 intervening region domain. With this mutation, the binding affinity of KEAP1 to the Nuclear Factor Erythroid-2-Like 2 (Nrf2) transcription factor, a master regulator of the antioxidant response, was increased[26]. Nrf2, which mediates the activation of cell-protective genes when released under normal conditions, may not be able to fulfill its task with the resulting mutation fully[26,27].

Another important recurrent hotspot change is the p.L861Q missense mutation in the EGFR gene in a case with p.W2686* nonsense mutation. This mutation occurs in the EGFR tyrosine kinase domain and has an oncogenic feature. Apart from these, TP53 p.V274F missense mutation (related ApoB mutation p.E2715* nonsense), TP53 p. G244V missense mutation (related ApoB mutation p. I3228Lfs*38 FS del), TP53 p. C176Y missense mutation and BRAF p. G469R missense mutation (related ApoB mutation p. E3303* nonsense) are oncogenic and may have a negative effect on tumor development in addition to changes in the ApoB gene. In addition, studies suggest that mutations in the ApoB gene are not directly oncogenic. Still, ApoB gene inactivation may be associated with overexpression of oncogenic regulators that support cancer development and downregulation of tumor suppressors[12,28]. This may suggest that ApoB may be involved in regulating the expression of oncogenic regulators and tumor suppressors and that its inactivation may contribute to the development and progression of cancer[28]. The fact that the most common recurrent hotspot mutation encountered in the above cases with the ApoB gene mutation is in the tumor suppressor gene TP53 may suggest that there may be a condition associated with the inactivation of the ApoB gene.

Formation of VLDL with ApoB-100 requires much energy. In tumor formation (the process of tumor formation), much energy is needed to support the rapid growth and proliferation of cancer cells. In this case, it is predicted that cancer cells may prefer ApoB-inactivating mutations. Because less ApoB-100 production will result in more energy for cancer metabolism[12], this hypothesis may suggest that inactivating mutations in the ApoB gene may promote cancer growth and progression by providing cancer cells with more energy.

In lung cancer, high ApoB levels have been associated with an increased incidence[6]. The results remain controversial, as lipoproteins containing ApoB have been implicated as risk factors for tumorigenesis. It is also thought that ApoB levels may be associated with a greater risk of cancer death[5]. Neither high nor low expression levels of the ApoB gene were associated with OS in the LUSC cohort. This may mean that changes in the ApoB gene do not affect survival from diagnosis (or initiation of treatment) to death.

The bioinformatics study states that the response to immune checkpoint inhibitors is weaker in cases with high ApoB expression levels[29]. It has been determined that ApoB is frequently mutated in hepatocellular cancer (HCC) patients and mutations in this protein account for almost 10% of all mutations observed in this patients[30]. The study investigating ApoB gene levels in HCC reported that ApoB gene ablation may be highly associated with poor clinical outcomes in HCC and proliferation of HCC cells[28]. While it has been reported that decreasing ApoB has a vital role in the development of breast cancer,[31] data from another study found that rs693 and rs1042031 polymorphisms in the ApoB gene increase the risk of breast cancer[32]. In addition, it was emphasized that the measurement of decreased ApoA1 levels and increased ApoB levels in patients with early-stage gastric cancer is a potential biomarker in the progression of the disease[33]. While the data on ApoB in different cancer types are in this direction, the expression levels of the ApoB gene in tumor tissue in the LUSC cohort and LUSC subtypes (basal, classical, primitive, and secretory) were lower than in healthy tissue, but this was not statistically significant. In this case, hypomethylation in the promoter region of the ApoB gene in the LUSC cohort may not have had a severe effect on ApoB gene expression levels. Moreover, in the first scenario, this cause of hypomethylation may be the result of posttranslational modifications. In the second scenario, it could be the result of global DNA hypomethylation.GlobalDNA hypomethylation is a hallmark of human cancer, but its functional consequences remain unclear. Gene expression

and DNA methylation are two important molecular processes that can be altered in LUSC, a type of lung cancer[34]. There are associations between DNA methylation alterations and various factors such as the specific types of lung cancer, mutations in genes known to drive cancer growth (such as KRAS, EGFR, and TP53), and major risk factors like sex, smoking status, and race/ethnicity[35]. By better understanding how DNA methylation is regulated and how different risk factors influence it, we can gain valuable insights into the process of carcinogenesis (the development of cancer). This knowledge can help develop cancer prevention strategies and personalized treatments targeting specific DNA methylation alterations.

CONCLUSIONS

Although severe mutations in the LUSC cohort impair ApoB gene function, the relationship between epigenetic changes in the ApoB gene, gene expression levels, OS, and LUSC is unclear. Therefore, ApoB needs to be validated for future studies to be a potential target for anti-cancer therapies and to evaluate ApoB mutation as a biomarker for cancer risk and prognosis. We also think the relationship between methylation and lung cancer should be investigated in depth with population-based studies. In this context, our study will shed light on future research.

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Ethical Approval: The data used in this study were obtained from the public database TCGA. Therefore, ethical approval was not required.
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