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Research Article

STK11 is a Potential Therapeutic and Prognostic Biomarker and Correlates with Immune Infiltrates in Non-Small Cell Lung Cancer

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Abstract

Objectives: This study aims to elucidate the role of Serine Threonine Kinase 11 (STK11) in non-small cell lung cancer (NSCLC), particularly its involvement in resistance to anti-PD-1 monoclonal antibody therapy in KRAS-mutated NSCLC. The study also explores the implications of STK11 alterations in prognosis, protein interactions, immune cell involvement, and drug sensitivity.

Methods: Comprehensive bioinformatic analyses were conducted to evaluate STK11 expression levels and mutational profiles in various NSCLC subtypes. The study correlated these findings with clinicopathological characteristics and assessed immune cell infiltration, immune microenvironment, and potential therapeutic options. Molecular docking analysis was also performed to investigate interactions with various inhibitors.

Results: The results reveal elevated STK11 expression across NSCLC, with a mutation rate of 14%, and an association with favorable prognosis. STK11 expression was found to correlate with immune cell infiltration and a cold immune microenvironment characterized by lower immune activity. Nutlin-3a (-) was identified as a potential therapeutic option for NSCLC cases with STK11 mutations. Molecular docking analysis provided insights into interactions with various inhibitors, offering perspectives for personalized therapeutic strategies.

Conclusion: This study underscores STK11 as a dual-faceted prognostic and therapeutic biomarker in NSCLC. The findings highlight the complex interplay between STK11 and immune activity, offering innovative avenues for tailored treatment approaches in NSCLC.

Keywords: Non-small cell lung cancer, STK11, immune cell infiltration, prognostic biomarker, therapeutic biomarker, Immunotherapy resistance

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Lung cancer remains a major public health problem. More than 1.8 million deaths from lung cancer are reported each year, making it the most common malignancy to cause death globally.[1] Despite decades of treatment advancement, the 5-year overall survival rate doesn't surpass 16%.[2] Non-small cell lung cancer (NSCLC) is the most common subtype of lung cancer, accounting for more than 85% of all cases, and includes lung adenocarcinoma

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(LUAD), lung squamous cell carcinoma (LUSC), and large cell carcinoma (LCC).[3]

Serine/threonine kinase 11 (STK11), also called liver kinase B1 (LKB1), was discovered as a new tumor suppressor gene only in 1998 by linkage analysis to Peutz-Jeghers syndrome (PJS).[4] STK11 has been identified as the major upstream kinase controlling AMP-activated protein kinase (AMPK). When metabolic homeostasis is compromised, the active STK11 complex directly phosphorylates AMPK to drive further downstream catabolic processes. It is also involved in several processes, including cell polarity, cell proliferation, apoptosis, and cell cycle arrest.[5] At normal energy levels, STK11/AMPK leads to negative inhibition of signaling pathways such as mTOR and MAPK, which, if left unchecked, may promote cancer.[6]

Alterations in the STK11 gene have been reported in several types of cancer. Its loss of function has been shown to alter the metabolism of cancer cells, allowing them to produce the energy needed for uncontrolled cell growth and proliferation more efficiently.[7] STK11 is the third most commonly mutated gene in NSCLC.[8] Poor overall survival (OS) and increased nodal or distant metastases have been associated with its mutations.[9]

It was reported that non-small cell lung cancer, especially lung adenocarcinoma, commonly harbors STK11 mutations, and 54% of these aberrations co-occur with KRAS mutations.[9] Patients with STK11 alterations have a worse prognosis than STK11-negative patients and are often resistant to immunotherapy.[10] Thus, a comprehensive analysis characterizing this gene is of major importance in order to circumvent immunotherapy resistance induced by STK11 mutations and target these patients with more effective therapeutic molecules.

In this study, we aim to highlight the important role of STK11 in NSCLC prognosis and emphasize its potential as a prognostic and therapeutic biomarker and to explore its interactions within the tumor cell microenvironment in NSCLC.

Methods

cProSite Database Analysis

Cancer Proteogenomic Data Analysis Site (cProSite) (https://cprosite.ccr.cancer.gov/) is a web-based interactive platform that provides online proteomics, phosphoproteomics, and genomics analysis for the Clinical Proteomic Tumor Analysis Consortium (CPTAC) data. Compared to regular analytical methods, cProSite offers faster online analysis, a more user-friendly environment, and less reliance on bioinformatics expertise.[11] In this study, cProSite was used to analyze STK11 expression levels in LUAD and LUSC.

UALCAN Database Analysis

To highlight the association between STK11 mRNA expression levels and clinicopathological characteristics in NSCLC, we used the UALCAN database (https://ualcan.path.uab.edu/). It is employed to easily explore, analyze, and visualize cancer data. UALCAN allows users to better understand genes, proteins, and signaling pathways affected by cancer.[12] The portal provides cancer researchers with the ability to analyze and access cancer transcriptome, proteomic, and patient survival data. Using data from The Cancer Genome Atlas (TCGA) project, UALCAN allows users to assess the expression of protein-coding genes and their impact on patient survival in 33 cancers. This resource includes TCGA microRNA (miRNA), long noncoding RNA (lncRNA), and promoter DNA methylation data, as well as mass spectrometry-based proteomics data from CPTAC. UALCAN provides easy access to precomputed analyses, including tumor subset-based gene/protein expression, promoter DNA methylation status, and Kaplan-Meier survival analysis.[12]

Cbioportal Database Analysis

The Cbioportal database (http://www.cbioportal.org) was utilized to investigate the mutational profile and prognostic significance of STK11. This database is an open-source resource that contains information obtained from The Cancer Genome Atlas (TCGA) (https://www.cancer.gov/tcga), which is a multidimensional cancer genomics dataset that allows the analysis of more than 400 terabytes of raw data about 33 different tumor types. The database comprises over 13,000 samples, and it allows for large-scale data processing, statistical analysis, and graphical representation of tumor changes from the gene to the protein level.[13,14]

Kaplan-Meier Plotter Analysis

The Kaplan-Meier Plotter (https://kmplot.com/analysis/) is an online tool that analyzes the correlation between gene expression and survival in 21 cancer types, including 6,234 breast, 2,190 ovarian, 3,452 lung, and 1,440 gastric cancer samples.[15] In this study, the Kaplan-Meier plotter was employed to investigate the association between STK11 expression and survival in NSCLC.

TIMER Database Analysis

To investigate the correlation between STK11 expression and immune cell infiltration in NSCLC, a gene module within the Tumor Immune Estimation Resource (TIMER) database (http://timer.cistrome.org/) was utilized. The TIMER database comprises 32 cancers from the TCGA dataset, encompassing 10,897 samples.[16] The TIMER database includes various types of immune cells, such as CD4+ T cells, CD8+ T cells, B cells, neutrophils, macrophages, and dendritic cells.

muTarget Database Analysis

muTarget (https://mutarget.com/result) is a platform designed to facilitate the identification of novel mutational targets. It aims to discover biomarkers and potential therapeutic targets in different solid tumors by linking somatic mutations to gene expression.[17] In this study, muTarget was used to identify the somatic mutations associated with altered STK11 expression in LUAD and LUSC.

STRING Database Analysis

To retrieve information about interacting genes or proteins, we utilized the Search Tool for the Retrieval of Interacting Genes (STRING) website (https://string-db.org/). The latest version of this database includes data on over 67 million proteins from 14,000 organisms.[18] In this study, we inputted STK11 into the STRING database to obtain information about its protein-protein interaction (PPI) network.

GeneMANIA Database Analysis

In order to establish a Protein-Protein Interaction (PPI) Network analysis for the STK11 gene, we utilized the GeneMANIA Cytoscape platform. It enables the construction of a composite gene-gene functional interaction network based on a gene list. The resulting network includes the genes that are most closely related to the original list, as well as functional annotations from the Gene Ontology. The edges in the network are annotated with details regarding the publication or data source from which the interactions were derived. The tool utilizes GeneMANIA's database of more than 1,800 networks, which includes over 500 million interactions across eight organisms, including A. thaliana, C. elegans, D. melanogaster, D. rerio, H. sapiens, M. musculus, R. norvegicus, and S. cerevisiae.[19]

GDSC Database Analysis

The Genomics of Drug Sensitivity in Cancer (GDSC) database, available at www.cancerRxgene.org, is the largest public resource providing information on drug sensitivity in cancer cells and molecular markers of drug response. The data comes from high-throughput screening performed by the Cancer Genome Project at the Wellcome Trust Sanger Institute and the Center for Molecular Therapeutics at Massachusetts General Hospital. The database offers freely accessible data on almost 75,000 experiments, covering reactions to 138 anticancer drugs across nearly 700 cancer cell lines.[20] In this study, we used the GDSC database to determine the potential drug sensitivity and selectivity of STK11 mutations. To visualize the results, we generated a volcano plot, an elastic network, and a scatter diagram.

Molecular Docking Analysis

The molecular docking analysis aimed to investigate interactions between potential inhibitors and the STK11 protein. The STK11 protein's crystal structure was sourced from the Protein Data Bank (PDB) and subsequently prepared by removing water molecules, heteroatoms, and co-crystallized ligands. Hydrogen atoms and charges were added to the protein using suitable force fields. Ligand structures were obtained from the PubChem compound database, then processed within OpenBabel GUI 3.1.1, facilitating 3D format conversion, assignment of atom types, and optimization of geometry while addressing structural issues. Molecular docking was executed utilizing AutoDock4 and AutoDock Vina, employing a search algorithm to explore ligand conformational space within the protein's binding site. Flexible docking of ligands into the receptor site allowed for the exploration of potential binding modes and prediction of binding affinity. Resultant docking poses were generated through multiple runs to account for ligand conformational flexibility. Docking results from AutoDock4 and AutoDock Vina were visualized and analyzed using Discovery Studio 2021, enabling 2D and 3D visualization of ligands and their interactions with amino acid residues, including van der Waals forces, hydrogen bonding, electrostatic interactions, conformational entropy, and desolvation terms, depicted through distinct bond colors.

Results

Pan-Cancer STK11 Gene Expression

In this study, we used the TIMER database to investigate STK11 expression alterations across multiple cancer types, including LUAD and LUSC. The analysis was conducted using publicly available gene expression datasets obtained from the TCGA database. Figure 1 demonstrates the relative expression levels of STK11 in various cancer types when compared to normal tissue samples. It is noteworthy that the majority of cancer types, such as breast invasive carcinoma (BRCA), cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), head and neck squamous cell carcinoma (HNSC), liver hepatocellular carcinoma (LIHC), prostate adenocarcinoma (PRAD), rectum adenocarcinoma (READ), and stomach adenocarcinoma (STAD), exhibit a significant increase in STK11 expression compared to normal tissues. However, while STK11 is expressed at notably higher levels in lung squamous cell carcinoma (LUSC), there is no significant differential expression of STK11 in the lung adenocarcinoma (LUAD) cohort.

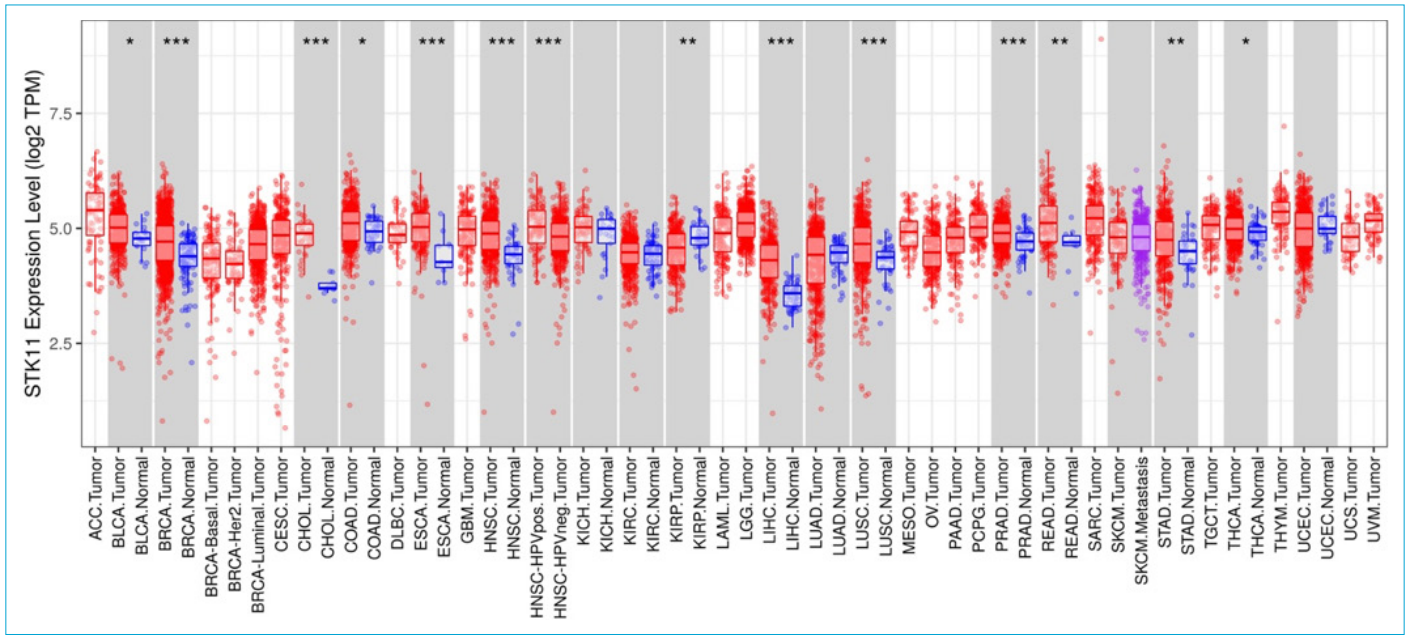

Figure 1. STK11 expression levels in different tumor types (*p<0.05, **p<0.01, ***p<0.001).

STK11 Gene Expression in LUAD and LUSC

We evaluated STK11 expression levels in LUAD and LUSC by utilizing the cProSite database. The graph depicted in Figure 2 displays the normalized expression values of STK11 mRNA in LUAD and LUSC samples, in comparison to underlying non-cancerous lung tissue. For lung adenocarcinoma, 213 samples were examined, of which 111 were cancerous. The findings reveal a significant upregulation of STK11 expression in LUAD samples when compared to adjacent non-cancerous lung tissues (p=0.0144). Similarly, in the case of squamous cell carcinoma, 108 cancer samples and 98 normal cells were analyzed. We observed a higher expression of STK11 mRNA in the cancer samples in comparison with normal tissue (p=0.0033).

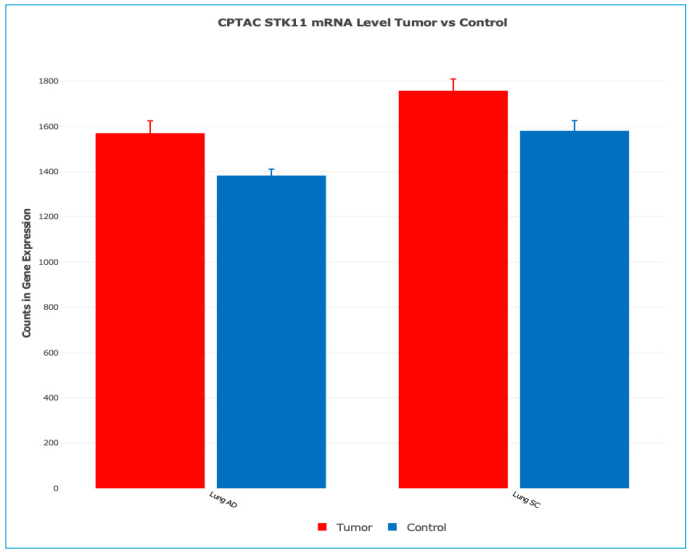

Figure 2. STK11 gene expression alteration in NSCLC subtypes.

We conducted additional analysis on the expression of the STK11 gene at the translational level in patients with LUAD and LUSC (Fig. 3). In the case of lung adenocarcinoma, a total of 194 samples, of which 94 were cancerous, were examined. For lung squamous cell carcinoma, 212 samples, with 102 being cancerous, were analyzed. Our findings revealed that the expression of the STK11 gene was significantly reduced in both cancer subtypes (p<0.0001).

Correlation between STK11 mRNA Levels and Clinicopathological Characteristics of NSCLC Patients

To highlight the correlation between STK11 mRNA levels and clinicopathological characteristics of NSCLC patients, we used the UALCAN database. We found that gender significantly correlated with altered STK11 expression. In fact, males (n=238) were associated with lower STK11 expression compared to females (n=276) (p<0.05 vs p<0.001, respectively). STK11 was underexpressed in the age group 21–40 years (n=12) compared to 41–60 years (n=90) (p=0.02), 61–80 years (n=149) (p=0.04), and 80–100 years (n=32) (p=0.03). STK11 was overexpressed in patients with N3 (n=2) (p=0.02) but not in N0 (n=331), N1 (n=96), and N2 (n=74). Our analysis showed no significant correlation between STK11 gene expression and patient's race, grade, smoking status, or tumor cell type (Fig. 4).

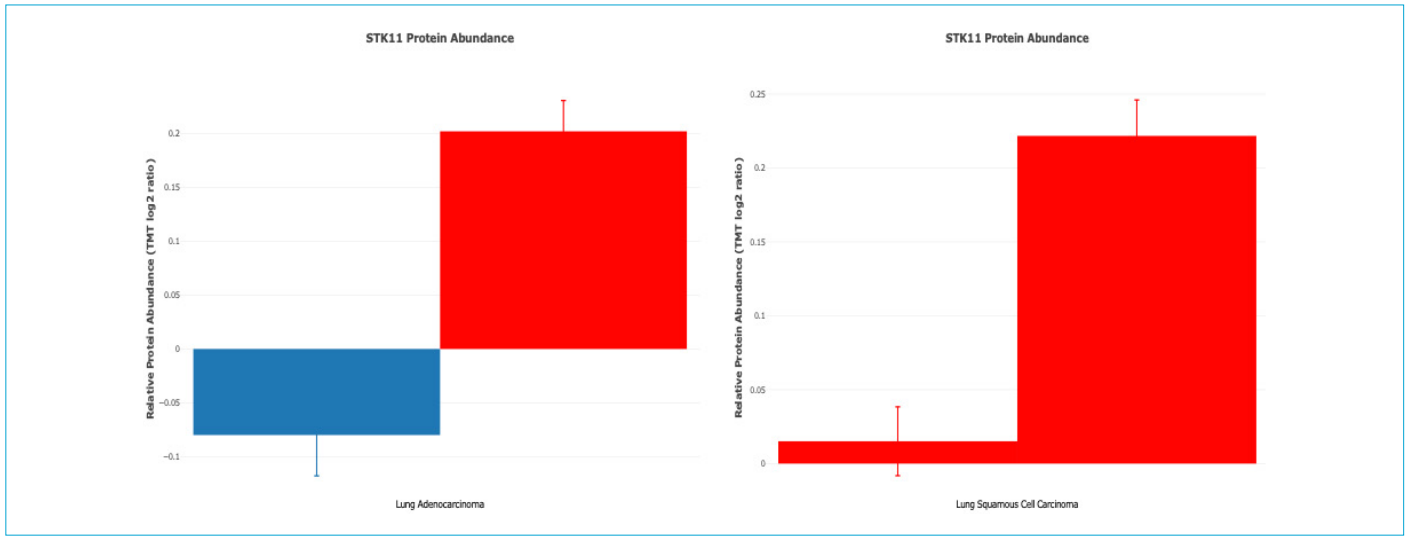

Figure 3. STK11 gene expression in lung adenocarcinoma and lung squamous cell carcinoma.

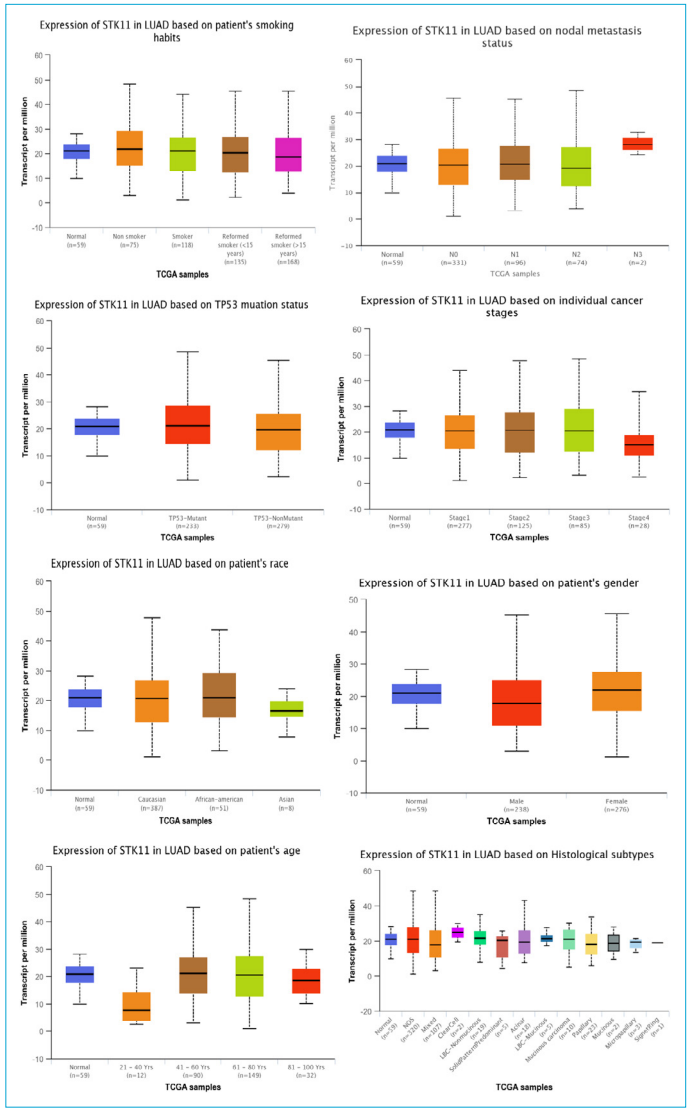

Figure 4. The correlation between STK11 expression and clinicopathological characteristics of LUAD patients.

The Mutational Profile of the STK11 Gene in LUAD and LUSC

To investigate the prevalence and spectrum of STK11 mutations in LUAD and LUSC, a comprehensive analysis was performed using data obtained from the cBioPortal database. The study focused on a cohort of LUAD and LUSC patients, and the lollipop graph was utilized to visualize the distribution of the mutations across different regions of the gene. Among the 11,927 samples that were analyzed, 12% (n=1,433) showed mutations in the STK11 gene. Among these mutations, the most common type was truncating mutations, accounting for 54.57% (n=782) of all the mutations. The second most prevalent type was missense mutations, making up 26.66% (n=382) of all STK11 mutations. Splice mutations followed with a proportion of 15.91% (n=228). The remaining 3% (n=41) of the mutations consisted of other types, such as nonsense mutations and in-frame site mutations (Fig. 5A).

The lollipop graph displayed the specific positions of the mutations within the STK11 gene, with each lollipop representing a kind of mutation and the height indicating the frequency of mutations at that particular position. The graph indicated a homogeneous distribution of STK11 mutations mostly located in the Pkinase region. Notably, the hotspot mutations were mostly located in the protein kinase domain. These hotspot regions displayed a higher frequency of mutations compared to other regions, suggesting potential functional significance. Several recurrent mutations were identified, including X97, X155, X200, S216, W239, G242, X245, G251, X288, and D194. D53Tfs/Gfs is the most frequent one and corresponds to a frameshift INDEL mutation located on exon 1 of the gene. These recurrent mutations highlight potential hotspots within the

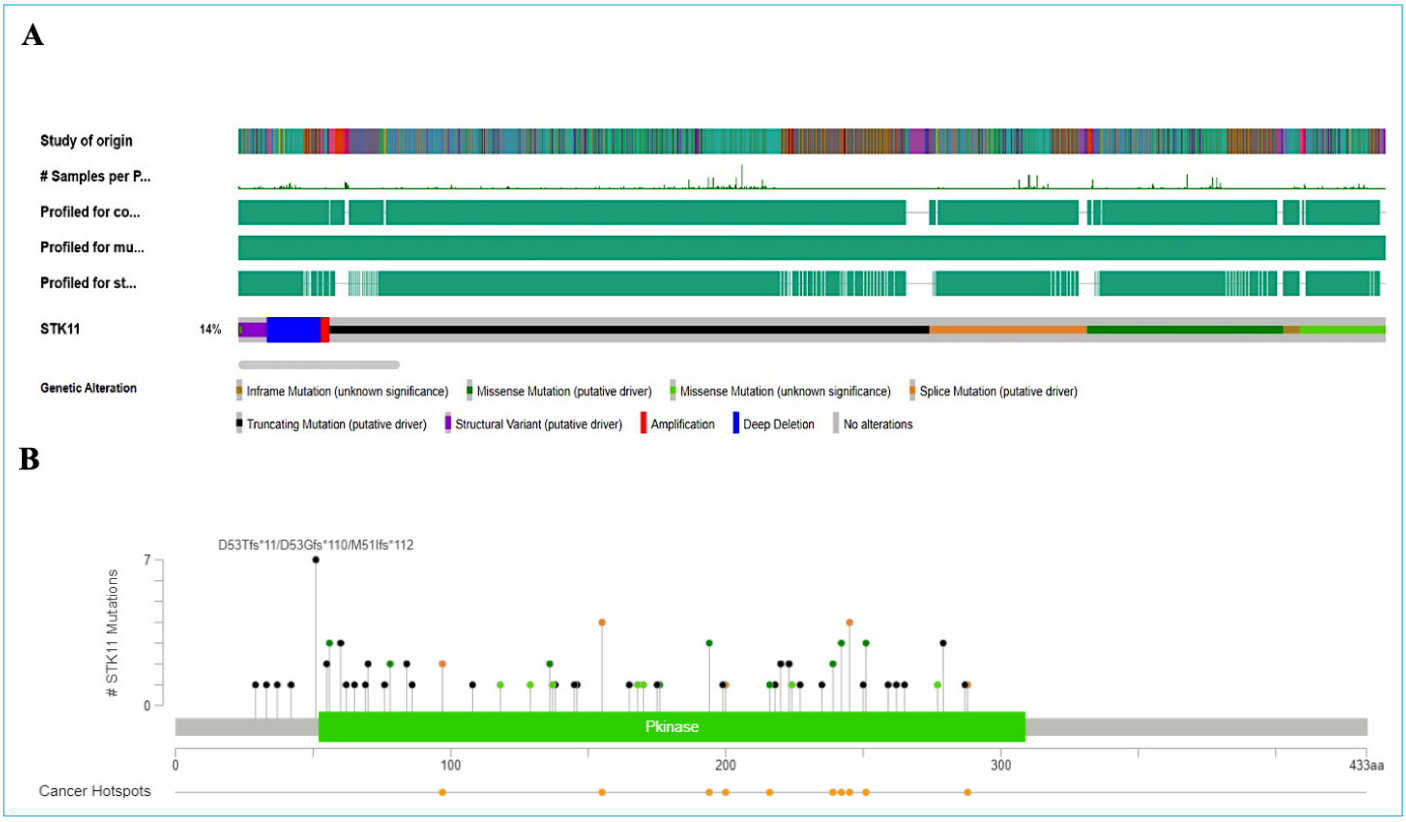

Figure 5. Frequency **(a)** and types **(b)** of STK11 mutations in non-small cell lung cancer patients.

STK11 gene that may be crucial in NSCLC development and progression (Fig. 5B).

The Prognostic Significance of the STK11 Gene in NSCLC

A univariate Kaplan-Meier analysis including a total of 1,411 NSCLC patients was carried out to assess the correlation between the level of expression of the STK11 gene and the overall survival rate (OS), post-progression survival (PPS), and progression-free survival (PFS). The high expression of STK11 showed a favorable prognosis (OS HR = 0.76, 95% CI=0.65-0.88, p=0.00019) (Fig. 6-A). Similarly, the progression-free survival result showed that the STK11 gene expression is significantly associated with a better prognosis in lung cancer (PFS HR =0.74, 95% CI=0.6-0.92, p=0.0069) (Fig. 6B). However, there was no significant correlation between gene expression and post-progression survival (p=0.46) (Fig. 6C).

We further analyzed the impact of STK11 expression on the overall survival of 979 patients using anti-PD1 therapy (Fig. 6D). The result shows that STK11 expression is significantly associated with a poor prognosis in lung cancer (OS HR=1.36, 95% CI=1.02-1.82, p=0.036), suggesting its implication in immunotherapy resistance observed in NSCLC patients.

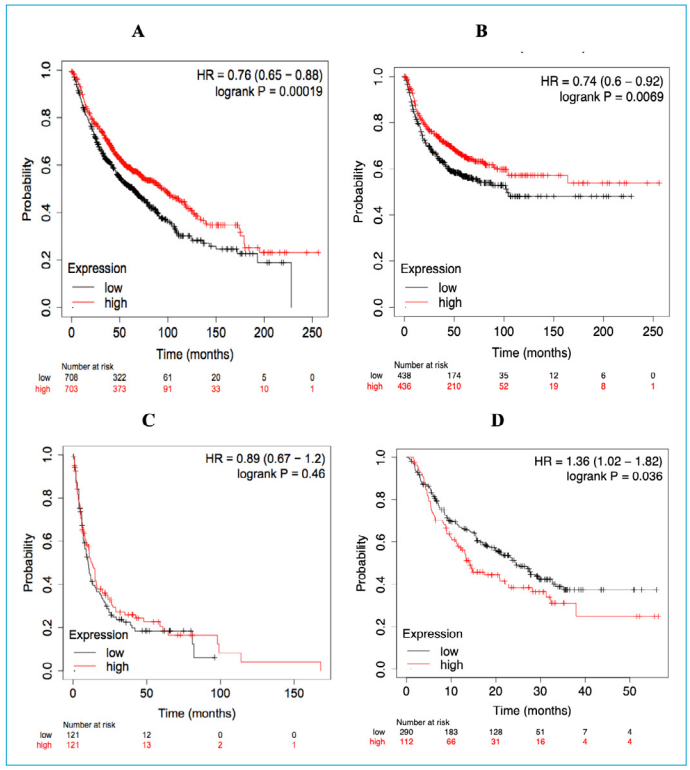

Figure 6. Kaplein-Meier overall survival curve **(a)**, progression-free survival **(b)**, progression post survival **(c)**, and anti-PD1 clinicopathological outcome **(d)**.

The Association Between STK11 Expression and Immune Infiltration

The tumor environment has a great impact on patient survival. In this study, we utilized the Tumor Immune Estimation Resource (TIMER) database to investigate immune cell infiltration in adenocarcinoma and squamous cell carcinoma. This analysis aimed to elucidate any potential associations between STK11 gene alterations and immune cell infiltration patterns in these types of cancers. The analysis focused on multiple immune cell types, including CD4+ T cells, CD8+ T cells, B cells, neutrophils, macrophages, and dendritic cells.

We first examined the correlation between STK11 expression and immune cell infiltration in adenocarcinoma samples. The TIMER analysis revealed distinct patterns of immune cell infiltration depending on STK11 expression status. Notably, STK11 gene expression was positively correlated with the level of immune infiltration of B cells (r=0.242, p<0.001), CD4+ T cells (r=0.491, p<0.001), neutrophils (r=0.215, p<0.001), macrophages (r=0.138, p<0.003), and dendritic cells (r=0.326, p<0.001). Additionally, a moderate decrease in CD8+ T cell infiltration was observed, although the difference did not reach statistical significance (p=0.119).

Similar to the adenocarcinoma analysis, we investigated immune cell infiltration in squamous cell carcinoma samples based on STK11 gene expression. The results showed a positive correlation with infiltrating levels of CD4+ T (p<0.001) but negative correlations with infiltrating levels of CD8+ T (p<0.001). However, there were no significant correlations with infiltrating levels of B cells (p 0.55), macrophages (p=0.169), neutrophils (p=0.853), and dendritic cells (p=0.973) in LUSC (Fig. 7). These findings indicate the immune cell landscape associated with STK11 gene alterations in LUSC, characterized by increased CD4+ T cell infiltration and a decrease in CD8+ T cell infiltration.

Co-mutated Genes Associated with Altered STK11 Expression in LUAD and LUSC

The somatic mutations associated with altered STK11 expression in LUAD and LUSC were identified using the muTarget platform. The results indicated that mutations in TRIP11 and ERICH6 were associated with elevated STK11 expression in LUAD, and mutated TOX2 and KCK10 genes were found to be associated with decreased STK11 mRNA expression. In LUSC, mutations in FOXD4LD, SMARCA2, PCDH1, or NLRP10 correlate with decreased STK11 mRNA expression (Fig. 8) (Table 1).

STK11 Protein–Protein Interactions Network

The STRING analysis was conducted to explore the protein-protein interactions network of STK11, a key tumor suppressor protein involved in various cellular processes. The aim of this analysis was to identify and visualize the potential interactions of STK11 with other proteins, shedding light on its functional associations and cellular pathways. The analysis yielded a list of the top ten proteins, along with their corresponding gene names (Fig. 9A). These genes include CAB39, CAB39L, STRADA, STRADB, HSP90AA1, AXIN1, TP53, CDC37, PRKAA1, and PRKAA2.

Furthermore, the GeneMANIA analysis revealed a comprehensive network of proteins associated with STK11 and their biological functions (Fig. 9B). The analysis was based on direct physical interactions, functional associations, co-expression patterns, shared protein domains, and genetic interactions. The biological functions carried out by STK11-related proteins include cell growth regulation, signaling pathways, and energy metabolism.

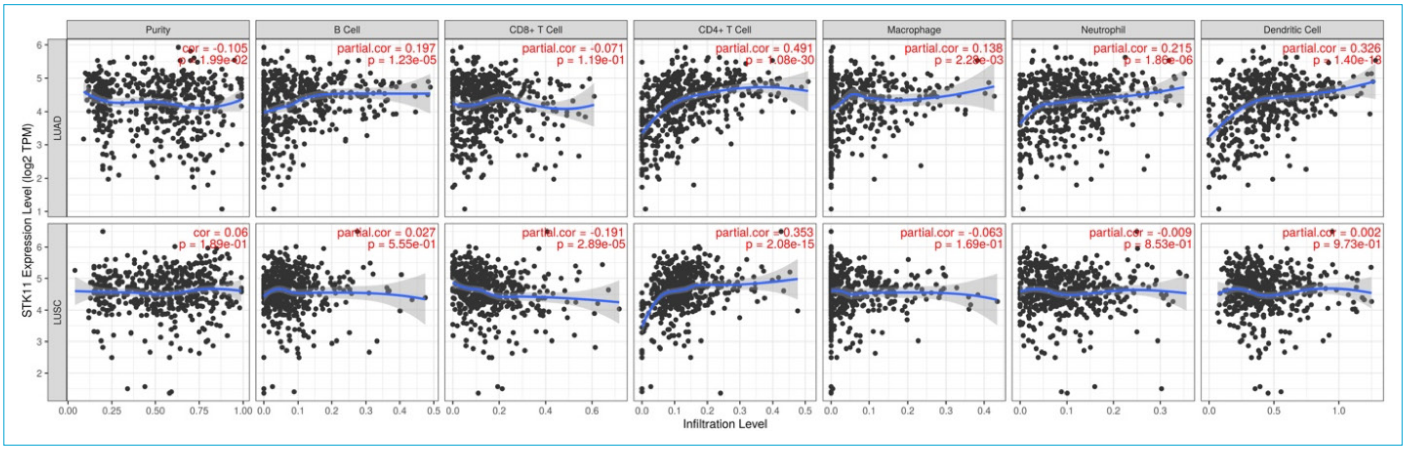

Figure 7. Correlation between STK11 expression and immune cells infiltration level in LUSC and LUAD.

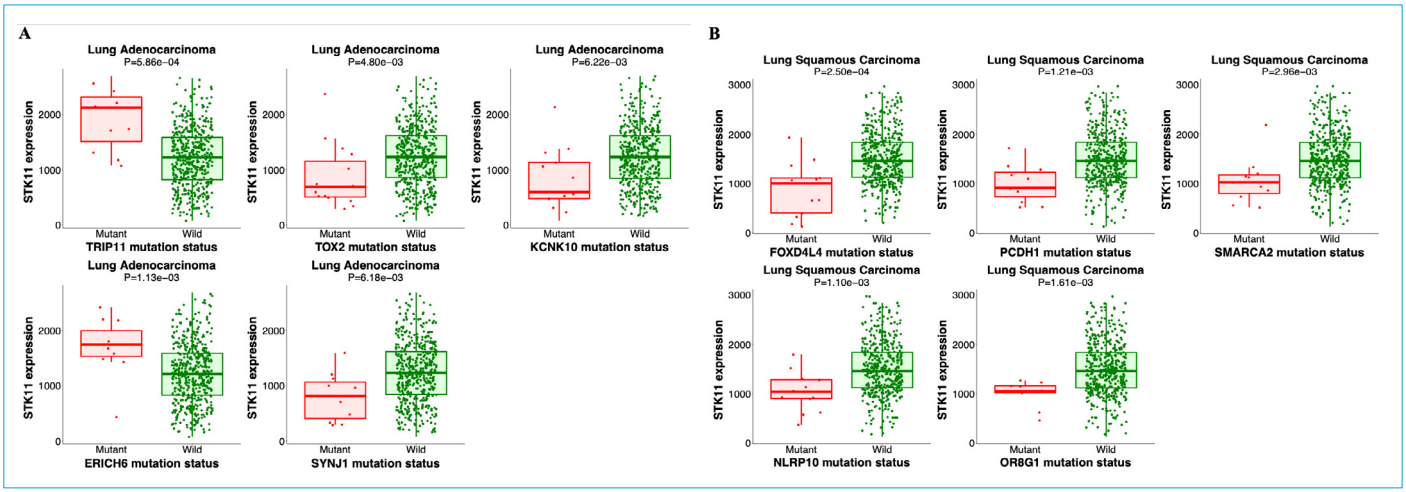

Figure 8. Five most mutated genes associated with altered STK11 expression in LUAD **(a)** and LUSC **(b)**.

Table 1. Top 8 most mutated genes associated with altered STK11 expression in LUAD and LUSC

Mutation of	Mean expression (mutant)	Mean expression (wild)	Number of mutant	Number of wild	FC (mutant/wild)	Direction	p
TRIP 11	1923.09	1235.98	11	497	1.56	Up	5.86e-03
ERICH 6	1833.67	1236.76	12	496	1.48	Up	1.13e-03
TOX2	862.4	1262.68	15	493	1.47	Down	4.80e-03
SYNJ1	801.27	1260.81	11	497	1.56	Down	6.18e-03
KCNK10	817.54	1262.24	13	495	1.54	Down	6.22e-03
RAB3GAP2	854.08	1261.28	13	495	1.47	Down	6.55e-03
TRPM7	1991.36	1234.47	11	497	1.61	Up	7.34e-03
LZTR1	834.83	1260.93	12	496	1.52	Down	7.75e-03
Mutation of	**Mean expression (mutant)**	**Mean expression (wild)**	**Number of mutant**	**Number of wild**	**FC (mutant/wild)**	**Direction**	**p**
FOXD4L4	876.62	1539.3	13	475	1.75	Down	2.50e-04
NLRP10	1034.38	1534.98	13	475	1.49	Down	1.10e-03
PCDH1	992.91	1533.84	11	477	1.54	Down	1.21e-03
OR8G1	1004.5	1532.47	10	478	1.52	Down	1.61e-03
SMARCA2	1055.64	1532.39	11	477	1.45	Down	2.96e-03
CERKL	1029.15	1535.13	13	475	1.49	Down	3.59e-03
SYT13	958.7	1533.42	10	478	1.59	Down	3.69e-03
CLEC4F	971	1534.35	11	477	1.59	Down	3.77e-03

The Signaling Pathways Regulated by STK11 Mutations

STK11-modulated signaling pathways were analyzed using the cBioPortal database (Fig. 10). The results show that STK11 tightly regulates cell growth by integrating signals related to energy status, nutrient availability, and growth factors.

STK11 Sensitivity to Therapeutic Molecules

Furthermore, we attempted to determine the potential drug sensitivity and selectivity of STK11 using the GDSC database. The purpose of this analysis was to identify possible associations between STK11 mutation status and tumor cell response to various anticancer drugs commonly used in the treatment of non-small cell lung cancer. GDSC screening results showed that STK11 exhibited high sensitivity to an undisclosed drug (720427) in all cancer types, including NSCLC. Additionally, STK11 mutant cells showed sensitivity to Nutlin-3a (-), especially in LUAD cases. Notably, this sensitivity has not been observed in other cancers with STK11 mutations. These results suggest that Nutlin-3a (-) may be a promising targeted therapy option for STK11-mutant LUAD patients and represent a potential new target for its treatment (Figs. 11,12,13).

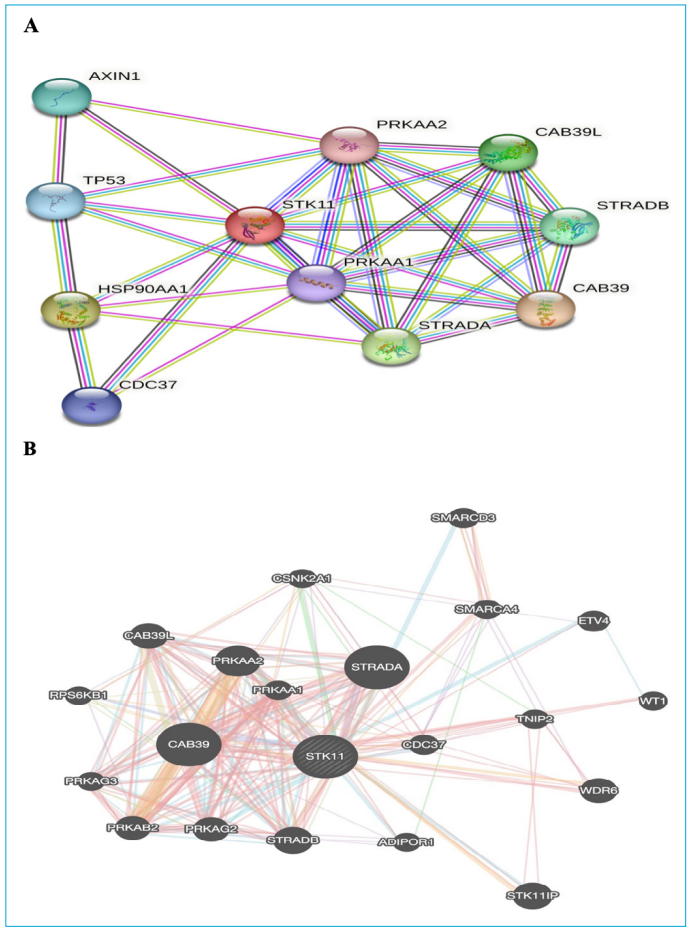

Figure 9. Protein–protein interaction network of STK11. **(a)** Protein–protein interaction network of STK11 analyzed by STRING. **(b)** Protein–protein interaction network of STK11 analyzed by GeneMANIA.

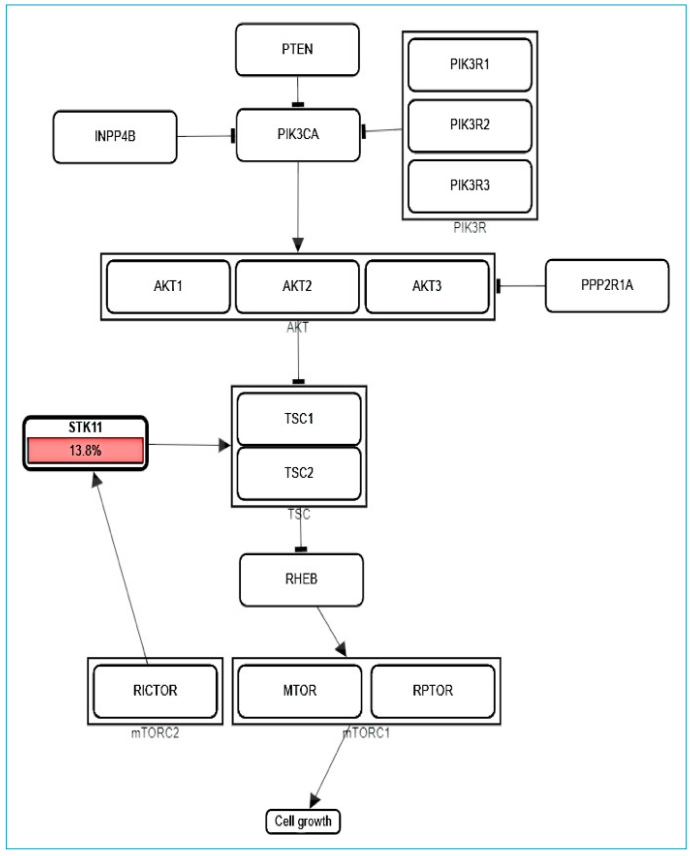

Figure 10. STK11-regulated signaling pathways.

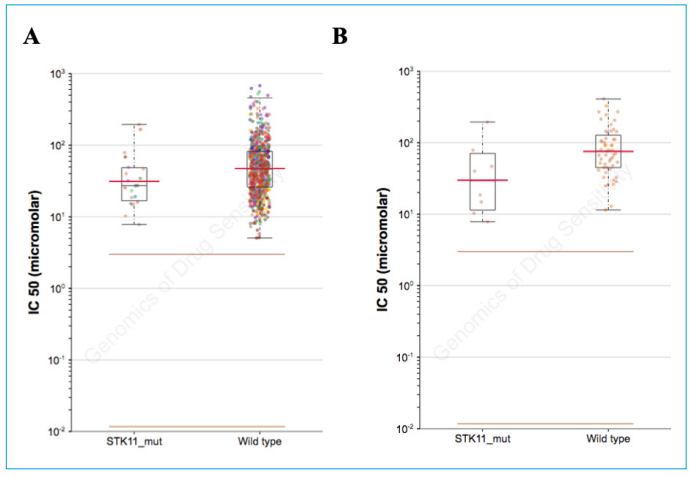

Figure 11. Scutter plot of 720427 drug effect in all cancer types **(a)** and NSCLC **(b)**.

Molecular Docking Analysis

In this docking study, we investigated the interaction between the STK11 protein and Nutlin 3A as our reference molecule and five inhibitors from the literature: Adagrasib, Sotorasib, Talazoparib, CB839, and Bemcentinib. The results confirmed the known interactions of Nutlin 3A with STK11 protein (score=-8.3) and provided additional evidence for the binding capabilities of Sotorasib (score=-8.6), Talazoparib (score=-8.6), CB839 (score=-8.9), and particularly Bemcentinib (score=-10.1) to efficiently target and modulate STK11 activity. Adagrasib also shows promise, although with a relatively moderate binding affinity (Fig. 14).

Discussion

The STK11 gene encodes liver protein kinase B1 (LKB1), an intracellular serine/threonine kinase that plays important roles in cell metabolism, cell polarity, regulation of apoptosis, and DNA damage response. Alterations in STK11 were found in 3.04% of cancers.[21] These alterations are mainly concomitant with KRAS mutations and are associated with poor prognosis.[22] Co-mutations of KRAS and STK11 genes have been reported to be associated with resistance to immune checkpoint inhibitors, especially anti-PD-1.[23]

In this study, we investigated STK11 expression levels in various types of tumors using the TIMER database. Our analysis revealed that the STK11 gene is highly expressed in various cancer types, including LUAD and LUSC. Similarly, the cProSite analysis showed that, compared to normal

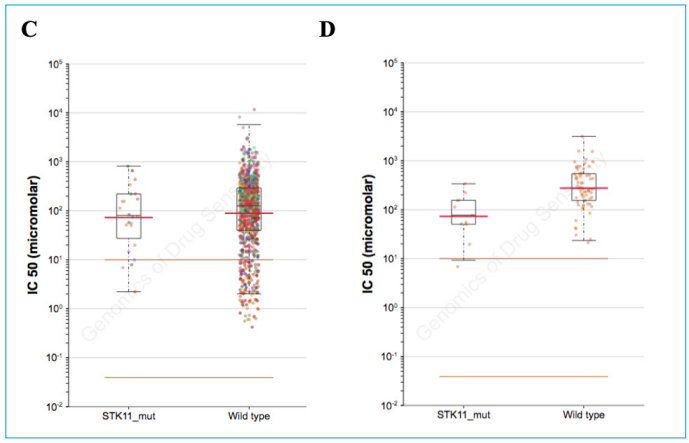

Figure 12. Scutter plot of Nutlin-3a (-) drug effect in all cancer types **(c)** and NSCLC **(d).**

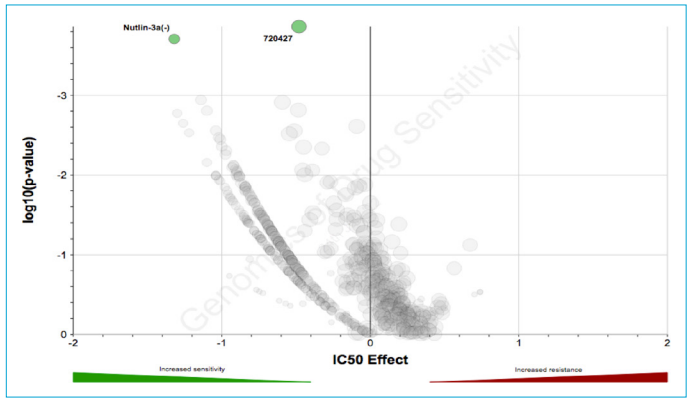

Figure 13. STK11 mutation and drug sensitivity.

tissues, STK11 mRNA expression was found to be higher in LUAD and LUSC. One study evaluated STK11 mRNA expression in the MCF-7 cell line of breast cancer and the MCF-10 cell line of normal breast cells using real-time PCR and found that the expression of STK11 mRNA was higher in cancer cells compared to normal cells ($p<0.0005$), indicating its potential role in the onset of breast cancer.[24] Another study reported that STK11 mRNA expression was lower in cholangiocarcinoma patients with mutant STK11.[25] All these findings suggest a potential implication of STK11 in tumorigenesis and progression, which motivates further investigation of its clinical significance.

The evaluation of STK11's prognostic value demonstrated a significant correlation between its expression level and

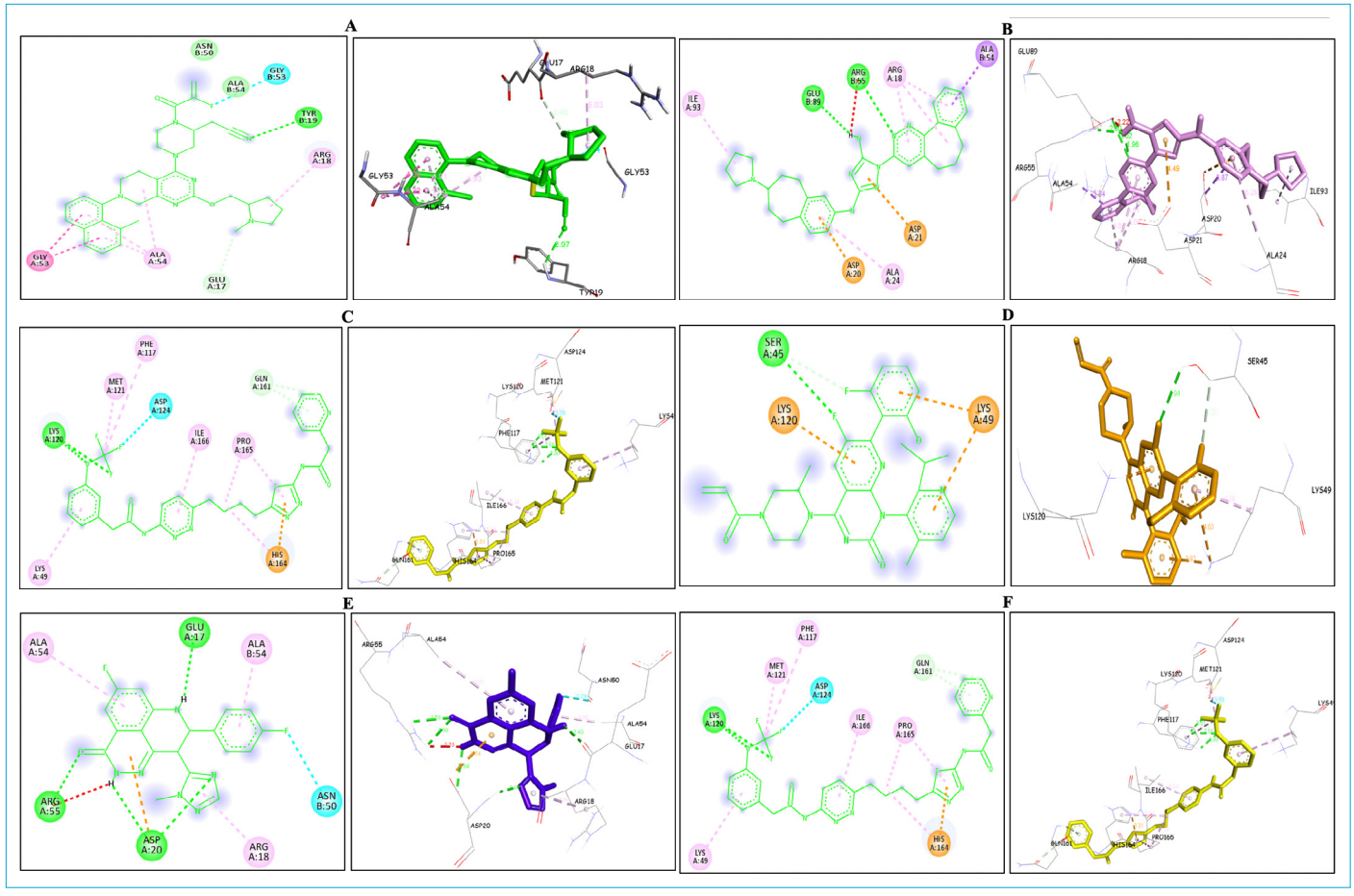

Figure 14. Representation (2D and 3D) docking results of best ranked pose of key interactions in the binding pocket of STK11 protein with Adagrasib **(a)**, Bemcentinib **(b)**, CB839 **(c)**, Sotorasib **(d)**, Talazoparib **(e)**, and Nutlin3a (-) **(f)**.

overall survival (p=0.00019), as well as progression-free survival (p=0.0069). The KM analysis indicates that higher STK11 expression is associated with improved survival outcomes. It was shown that controlling the energetic response to cellular stress has been identified as a significant vulnerability to STK11-deficient tumors. Tumors lacking STK11 are extremely vulnerable to apoptosis due to the inability to restore ATP levels.[26] Nonetheless, the available evidence suggests that STK11 gene expression alterations are generally associated with a poor prognosis in cancer patients. For example, STK11 alterations have been linked to worse outcomes in the pan-cancer context, regardless of therapy, with KRAS and TP53 being the most commonly co-altered genes.[27] While there is no study to suggest that STK11 gene mutations are associated with a better prognosis in cancer patients, further research is needed to fully understand the clinical implications of STK11 alterations in different cancer types. Additionally, the UALCAN analysis showed that STK11 expression was significantly associated with patient's gender, age, and nodal metastasis status. Taken together, these findings suggest that STK11 is a potential prognostic biomarker of NSCLC.

Another important finding of our study is that STK11 expression influences the immune checkpoint inhibitor response. According to the KM analysis, STK11 expression was associated with poorer OS outcomes when treated with immune checkpoint inhibitors. It was demonstrated that low levels of PD-L1 expression were observed in patients with STK11 mutations, which could explain immunotherapy resistance.[28] Nonetheless, it was reported that patients with STK11-mutated tumors showed a better response to immunotherapy, particularly when co-occurring with TP53 mutations.[29]

Increased immune cell infiltration into cancers improves patient survival and predicts sensitivity to immune treatments.[30] Thus, identifying the factors controlling immune cell infiltration levels is critical for developing strategies to intervene on these targets. Results obtained using the TIMER database show that in both LUAD and LUSC, CD8+ T cells are highly and negatively associated with altered STK11 expression. It has been reported that CD8+ T cells influence the response to ICIs.[31] Furthermore, a similar study using the TIMER database on LUAD showed that the STK11 mutation group had reduced immune cell infiltration in B cells, CD8+ T cells, CD4+ T cells, macrophages, and dendritic cells. This entails that LUAD harboring STK11 mutations can cause the "cold" tumor immune microenvironment.[32] Taken together, these findings suggest that STK11 potentially regulates immune infiltrating cells' recruitment and regulation, and therefore influences NSCLC prognosis.

It was demonstrated that STK11 functions as a kinase, yet its role in regulating NSCLC remains unclear. Therefore, we examined the protein-protein interaction network of STK11 and co-expressed genes in both LADC and LUSC tissues. Furthermore, we used STRING and GeneMANIA databases to illustrate interactions between STK11 and other partners. The proteins related to STK11 are implicated in cell growth regulation, signaling pathways, and energy metabolism. The STK11-modulated signaling pathway depicted using cBioPortal showed that STK11 tightly regulates cell growth by integrating signals related to energy status, nutrient availability, and growth factors. STK11 acts as a major regulator of energy sensing and cellular metabolism, rescheduling cell metabolism, slowing down cell growth and ATP-consuming processes, as well as promoting cell survival under stress conditions.[6] This process can be thought of as a cellular stress-activated "checkpoint" that prevents certain cellular activities, such as protein translation or cell division, from occurring under suboptimal conditions. Cancer cells may use the same mechanism to meet their nutritional needs when under stress.[27]

Despite the advancements in lung cancer treatment, the survival rate among patients remains poor.[33] Examination of the TCGA database of LUAD patients with STK11 mutations indicated that treatment approaches such as chemotherapy can significantly improve the survival rate of NSCLC patients with STK11 mutations.[34] We used the GDSC database to search for efficient and selective drugs against STK11-mutant NSCLC. According to screening results, an undisclosed drug (720427) was shown to be efficient against STK11-mutated tumors. Additionally, Nutlin-3a (-), a small molecule inhibitor that targets the p53-Mdm2 interaction, was demonstrated to be potentially effective and selective against STK11-mutated LUAD.[35] Through our screening, we found that there has been minimal progress in identifying therapeutic compounds that target STK11 alterations, and additional research is needed to address this issue.

The results of the docking analysis of STK11 interactions with Nutlin 3A and four potential inhibitors from the literature (Adagrasib, Sotorasib, Talazoparib, and CB839) emphasize the importance of considering STK11 gene mutations as potential biomarkers for targeted therapies in NSCLC. Some drugs may be effective regardless of STK11 status, whereas others show promising potential as targeted therapies in combination with immunotherapies for STK11-mutated NSCLC. Thus, the development of therapeutic molecules targeting STK11 alterations has the potential to improve patient outcomes in cancer treatment, particularly in overcoming immunotherapy resistance in NSCLC and other cancer types with a high prevalence of

STK11 mutations. Further research and clinical trials are needed to fully understand the clinical implications of STK11 alterations and to develop effective therapies targeting these variations.

Conclusion

The in-silico analysis of the STK11 gene in NSCLC has revealed the potential significance of the STK11 gene in non-small cell lung cancer. Our results suggest that STK11 is a potential independent prognostic biomarker for NSCLC and can influence the treatment choice. We have shown that STK11 is overexpressed in both LUAD and LUSC, and its upregulation is associated with better overall survival in both cancer subtypes. We have further demonstrated that most hotspots are mainly located in the protein kinase region, and the STK11 gene expression significantly correlated with the infiltration of immune cells in LUAD and LUSC, which holds a predictive value for the response to immunotherapy.

Disclosures

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