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ORIGINAL ARTICLE

AKT2 for Modifying the Tumor Immune Microenvironment in Lung Adenocarcinoma

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SUMMARY

Background: **The phosphatidylionsitol 3-kinase (PI3K)-v-akt murine thymoma viral oncogene homolog (AKT)-mammalian target of rapamycin (mTOR) pathway has been extensively studied in lung adenocarcinomas (LUAD). This study aimed to explore the correlation between this pathway and the tumor microenvironment.**
Methods: **Data from the Cancer Genome Atlas (TCGA) was utilized to analyze variations in the expression of v-akt murine thymoma viral oncogene homolog 2 (AKT2) between LUAD tissues and normal tissues and to assess its effect on the survival of patients. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analysis were performed. The "R language" was used to analyze the disparity between immune cell infiltration in tumor tissues and the correlation with AKT2 expression levels.**
Results: **AKT2 was significantly upregulated in LUAD. The high expression level of AKT2 was significantly associated with shorter overall survival. Gene Set Enrichment Analysis revealed that tumor immune-related pathways such as adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin super-family domains were more active in the AKT2 high expression group. Tumor tissues with high AKT2 expression tended to have higher levels of regulatory T cells (Tregs) and CD8+ T cells and lower levels of activated dendritic cells and γδT cells. AKT2 expression was positively influenced by common immune checkpoints and correlated with TMB, suggesting that high AKT2 expression in LUAD may lead to significant immune evasion.**
Conclusions: **Tumor microenvironment in high AKT2 expression patients demonstrated immunosuppressive characteristics such as reduced γδT cells aggregation and increased Tregs infiltration.**
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KEYWORDS

AKT2, lung adenocarcinomas, Treg cells, γδT cells, tumor microenvironment

LIST OF ABBREVIATIONS

AKT - v-akt murine thymoma viral oncogene homolog
AKT2 - v-akt murine thymoma viral oncogene homolog 2
CAR-T - Chimeric antigen receptor T-cell immunotherapy
CC - Cellular component
CPS - Combined positive score
CTLA-4 - Cytotoxic T lymphocyte-associated antigen-4
GO - Gene Ontology
GSEA - Gene Set Enrichment Analysis
HLA-1 - Human leukocyte antigen class 1 antigen
ICIs - Immune checkpoint inhibitors
IHC - Immune histochemistry
KEEG - Kyoto Encyclopedia of Genes and Genomes
LAG3 - Lymphocyte activation gene-3
LUAD - Lung adenocarcinoma
MF - Molecular function
MSI-H - Microsatellite instability-high
mTOR - Mammalian target of rapamycin
NGS - Next-generation sequencing
NSCLC - Non-small cell lung cancer
PIK3CA - Phosphoinositide-3-kinase, catalytic, alpha polypeptide
PIK3R1 - Phosphoinositide-3-kinase, regulatory subunit 1
PI3K - Phosphatidylionsitol 3-kinase
TCGA - The Cancer Genome Atlas
TCR-T - T-cell receptor engineered T cell therapy
TIL - Tumor infiltrating lymphocyte
TMB - Tumor mutation burden
ZSC2 - Tuberous sclerosis 2

INTRODUCTION

Lung cancer is the most prevalent malignant tumor with the highest incidence and mortality rate globally, with lung adenocarcinomas (LUAD) emerging as a significant pathological subtype of non-small cell lung cancer (NSCLC). At present, immunotherapy, represented by anti-programmed cell death protein 1/programmed cell death ligand 1 (anti-PD-1/L1) and anti-cytotoxic T lymphocyte-associated antigen-4 (anti-CLTA-4) antibodies, has become the standard of care for NSCLC without driver gene mutations. Monotherapy administered to patient populations expressing PD-L1 or combined with chemotherapy for the all-comer population can extend the overall survival (OS) of patients [1,2]. Despite these advancements, some patients show limited responsiveness to immunotherapy, underscoring the urgent need to address this clinical challenge. The development of next-generation tumor immunotherapies aims to enhance survival rates and combat drug resistance. The utilization of biomarkers to identify cohorts with potential benefits is one of the important research directives. Furthermore, an essential strategy involves transforming "cold tumors" into "hot tumors" by modifying the tumor immune microenvironment, that is, transforming the immune-excluded and immune-desert form into the immune-inflamed phenotype [3].

Based on these two research directions, researchers are constantly exploring potential combination therapies that could enhance the anti-tumor activity. At the same time, in recent studies, the focus has been on the impact of the tumor microenvironment on the efficacy of immunotherapy. Based on the latter, some novel immunotherapies, cell therapies such as tumor infiltrating lymphocytes (TILs), T-cell receptor-engineered T cell therapy (TCR-T), and chimeric antigen receptor T-cell immunotherapy (CAR-T) have also become the focus of current studies [4].

The aim of this study was to identify biomarkers or targets that may have an effect on the tumor immune microenvironment by analyzing the LUAD dataset from the Cancer Genome Atlas (TCGA) database. Through a comparative analysis of immune status variations within the tumor microenvironment at different target gene expression levels, the goal was to determine if these variations hold potential as targets for enhancing the anti-tumor activity of immunotherapy.

MATERIALS AND METHODS

Data and information collection

RNA-seq data and clinical information of 549 individuals diagnosed with LUAD, encompassing both adenomas and adenocarcinomas, were obtained from the TCGA-LUAD project via the official TCGA database website (https://portal.gdc.cancer.gov/). The dataset includes data from individuals of diverse ethnic backgrounds.

Differential analysis of key genes expressions in PI3K/AKT/mTOR pathway

The comparative analysis of several key genes, including v-akt murine thymoma viral oncogene homolog 1 (AKT1), AKT2, mTOR, phosphoinositide-3-kinase, catalytic, delta polypeptide (PIK3CD), phosphoinositide-3-kinase, regulatory subunit 1 (PIK3R1) and phosphoinositide-3-kinase, catalytic, alpha polypeptide (PIK3CA) expression levels in the PI3K/AKT/mTOR pathway between tumor tissues and normal tissues in LUAD was conducted using TIMER 2.0 (http://timer.cistrome.org/Cbioportal). The "ggboxplot" package and "stat_compare_means" function of R language (R 4.3.1) were used to analyze the difference in these key gene expressions between LUAD tumor tissues and normal tissues. The "ggpaired" package was used for pair-wise differential analysis of key gene expression between tu-

mor tissues and corresponding precancerous normal tissues. The difference in the expression of protein levels of these genes between LUAD tumor tissues and normal tissues was compared using the Human Protein Atlas (HAP) database (https://www.proteinatlas.org/).

Survival prognosis analysis

The stage IIIB-IV LUAD cases were divided into high expression and low expression groups based on the expression level of differential genes selected from differential analysis of key genes expressions in PI3K/AKT/mTOR pathway. The OS of the two groups were compared using the "survminer" package of R language. Additionally, univariate and multivariate independent prognostic analysis were conducted to evaluate the prognostic effect of these genes on LUAD. Receiver operating characteristic (ROC) curves were generated using the "timeROC" package to understand the predictive effect of key differential gene expressions on 1-, 3-, and 5-year OS rates.

Co-expression genes analysis

The high/low expression of AKT2 in tumor tissues was compared with other genes using "limma" package to explore the co-expression of AKT2 with other genes and visualize to generate a heatmap. Furthermore, RNA-seq data were used to analyze the co-expression of key genes in PI3K-AKT-mTOR pathway with AKT2 to understand the positive and negative regulatory relationships between AKT2 and other genes in this pathway.

GO, KEGG, and GSEA analyses

Co-expression analysis was conducted using the "clusterProfiler" package of R language to investigate genes that positively and negatively regulate the expression of AKT2 in tumor tissue samples. The samples were divided into high/low groups based on the AKT2 expression level. Gene Set Enrichment Analysis (GSEA) was conducted, including Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. The GO analysis comprised of biological process (BP), molecular function (MF), and cellular component (CC). This comprehensive methodology was designed to examine the enrichment of various functions and pathways in the high/low AKT2 expression groups and to assess the impact of gene expression changes on biological pathways.

Immuno-infiltration analysis

The "stat_compare_means" function of R language was used to compare the immune cell content of tumor samples and precancerous tissues. This analysis facilitated the investigation of immune cell infiltration in tumor tissues with varying AKT2 expression levels and enabled the analysis of the correlation between different immune cell infiltrations and target gene expression levels. The "ggboxplot" was used to generate box plots, and the "ggplot" was used to generate scatter plots for visualization.

Immunotherapy efficacy prediction

The correlation between the target gene in tumor tissue samples and the expression level of common immune checkpoint genes was explored using immune checkpoint correlation analysis. The genes with a value of $p \leq 0.001$ in the correlation test were selected and visualized using the "corrplot" function. Spearman's correlation analysis was used to compare the correlation between target genes and TMB in tumor tissue samples, to predict immunotherapy efficacy.

RESULTS

Key genes in the pathway are significantly overexpressed in LUAD

Based on the analysis conducted using TIMER 2.0, a notable disparity in key genes, including PIK3CA, PIK3CD, PIK3R1, AKT1, AKT2, and mTOR expression levels between tumor tissues and normal tissues in LUAD indicated that the expression of AKT2, mTOR, PIK3CA and PIK3R1 was significantly different ($p < 0.01$) (Figure 1). Specifically, a comparative analysis of these gene expressions in 503 cases of tumor tissues and 54 cases of precancerous normal tissues in patients with LUAD revealed that the upregulation of AKT2, mTOR, and PIK3CA and the downregulation of PIK3R1 was pronounced, demonstrating a significant difference ($p < 0.05$). No significant difference of PIK3CD was detected (Figure 2a). Furthermore, a pair-wise comparative analysis in 54 patients both with tumor tissues and precancerous normal tissues revealed that AKT2 and mTOR were significantly upregulated and PI3KR1 was significantly downregulated in tumor tissues ($p < 0.001$) (Figure 2b). To verify this trend, the immunohistochemical staining results of AKT2 in LUAD were obtained through the HAP database. The staining of AKT2 in LUAD was mostly strongly positive or moderately positive, while its expression in normal tissues was low or negative. The abovementioned analysis suggests that the increase of AKT2 and mTOR expression or the decrease of PIK3R1 expression may have a significant correlation with the development of LUAD.

High expression of AKT2 is associated with unfavorable survival prognosis

The dataset comprising cases categorized as stage IIIB-IV LUAD was divided into two groups based on the AKT2, mTOR, and PI3KR1 expression levels: a high expression and a low expression group. The Kaplan-Meier curves were used to analyze the survival data of the two groups with varying gene expression levels. The results of the OS analysis were generated and compared and revealed that there was no significant difference between groups exhibiting high and low mTOR or PI3KR1 expression levels (Figure 3a). Meanwhile, a

Table 1. Correlation analysis between AKT2 and key genes in PI3K-AKT-mTOR pathway.

	Gene	Correlation	p-value
AKT2	MTOR	0.18	3.12×10^{-10}
AKT2	PIK3CD	0.20	8.86×10^{-6}
AKT2	TSC2	0.33	5.81×10^{-14}
AKT2	GSK3A	0.58	9.04×10^{-46}
AKT2	PIK3R1	-0.17	1.54×10^{-4}
AKT2	PTEN	-0.25	1.23×10^{-8}

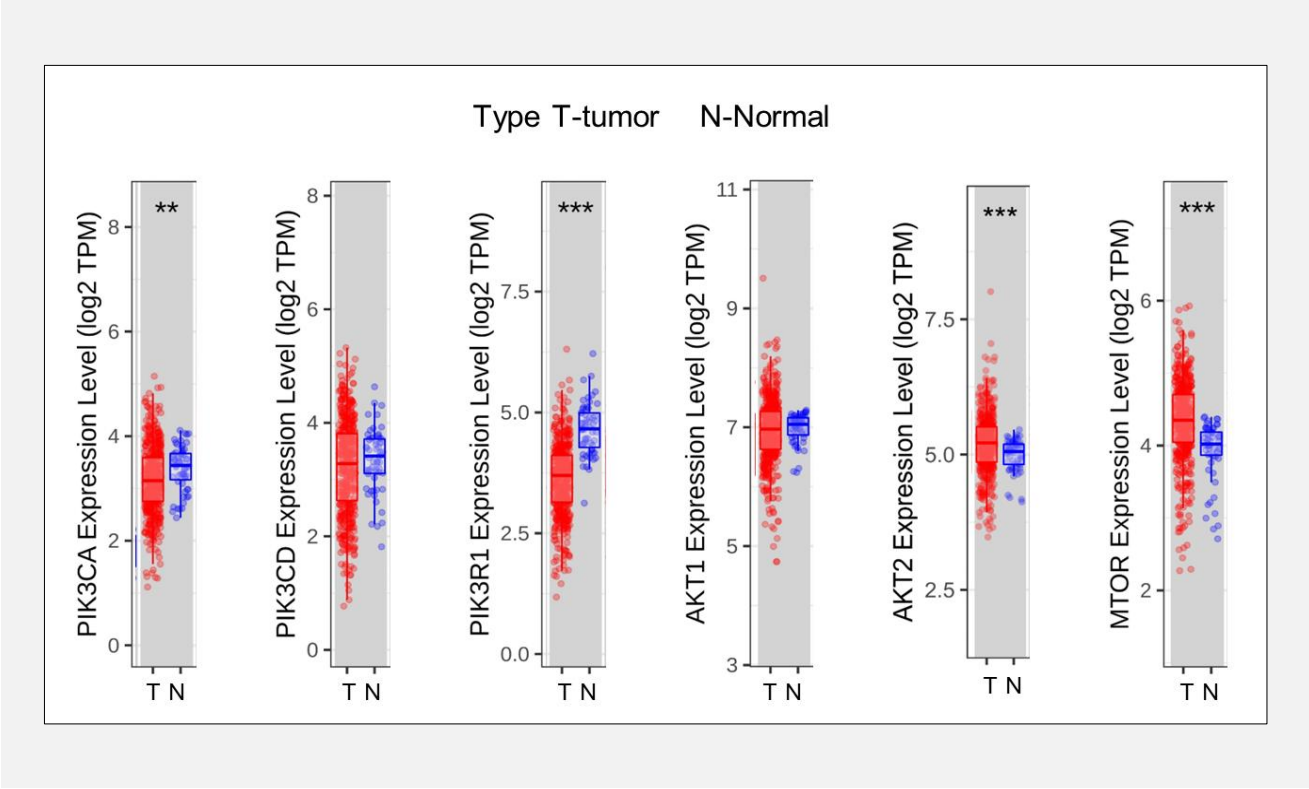

Figure 1. Differential analysis of key gene expression in tumor tissues and precancerous normal tissues of LUAD (* p < 0.05, ** p < 0.01, *** p < 0.001).

difference was seen between high and low AKT2 expression groups. The OS in the AKT2 high expression group was significantly lower than in the low expression group ($p < 0.05$) (Figure 3a). Furthermore, when the expression level of AKT2 was used to predict the 1-, 3-, and 5-year OS rates of patients with LUAD, it was discovered that the area under the curve (AUC) of the 5-year survival rate exceeded 0.7. This indicates that the expression level of AKT2 has a favorable predictive effect on the survival of patients with LUAD (Figure 3b). Univariate and multivariate independent prognostic analyses revealed that the survival rate of patients was significantly correlated with the AKT2 expression level ($p < 0.001$) (Figure 3c, 3d).

Co-expression genes analysis results

An analysis was conducted to assess the genes that influence the expression of AKT2 in tumor tissue samples, revealing positive correlations with mTOR, PIK3CD, tuberous sclerosis 2 (TSC2), and glycogen synthase kinase 3 alpha (GSK3A) genes and negative correlations with PIK3R1 and gene of phosphate and tension homology deleted on chromosome ten (PTEN). These genes exhibited clustering within the PI3K-AKT-mTOR pathway (Table 1) [5].

The genes co-regulatory relationship with AKT2 was further generated and visualized in a heatmap (Figure 4). There were 313 genes with a significant correlation with the expression of AKT2. Among them, 110 genes

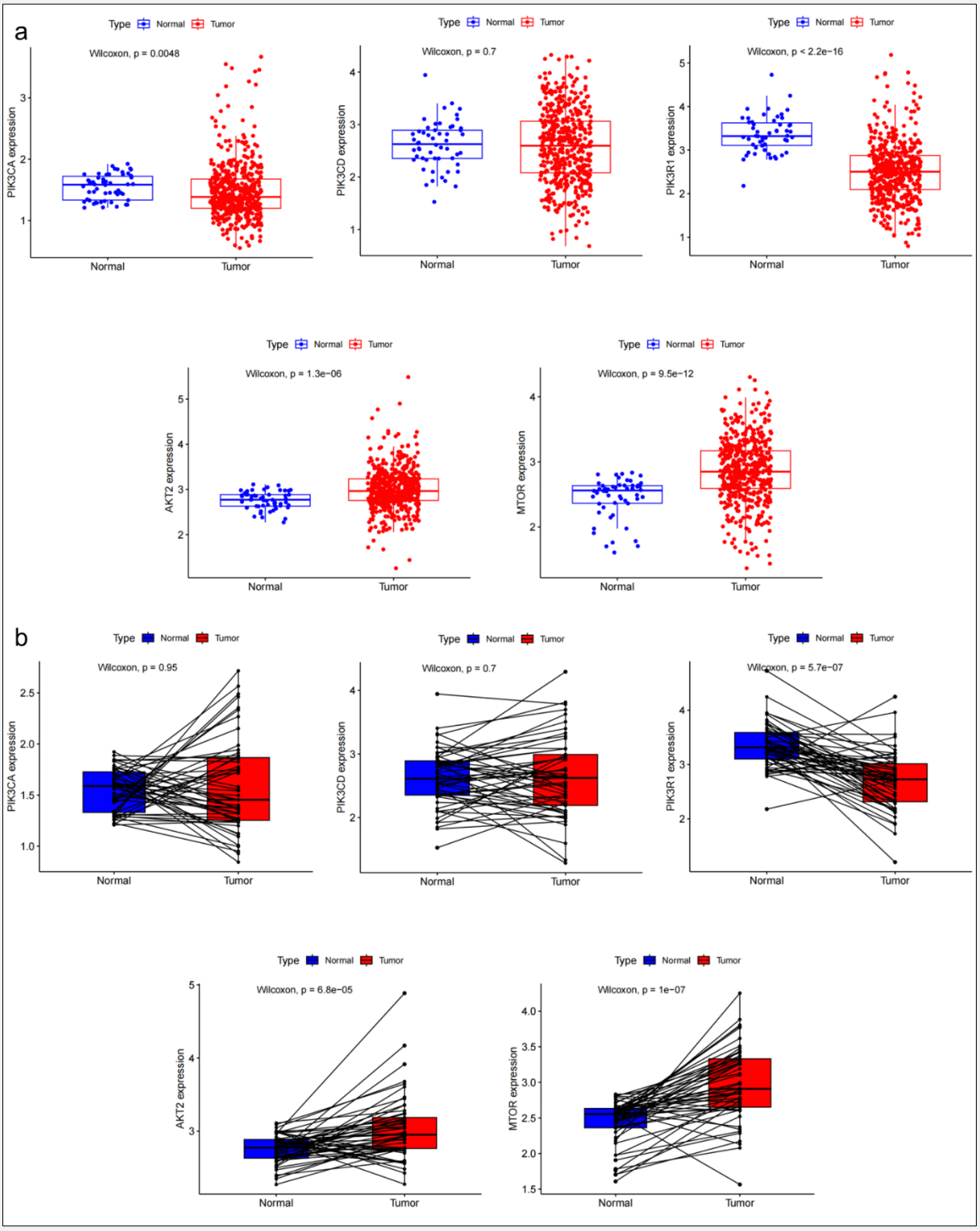

Figure 2. Key gene analysis in LUAD tumor tissue and normal tissue.

a) Differential analysis of key genes in tumor tissues and precancerous normal tissues of LUAD.
b) Pairwise differential analysis of key genes in tumor tissues and precancerous normal tissues of LUAD.

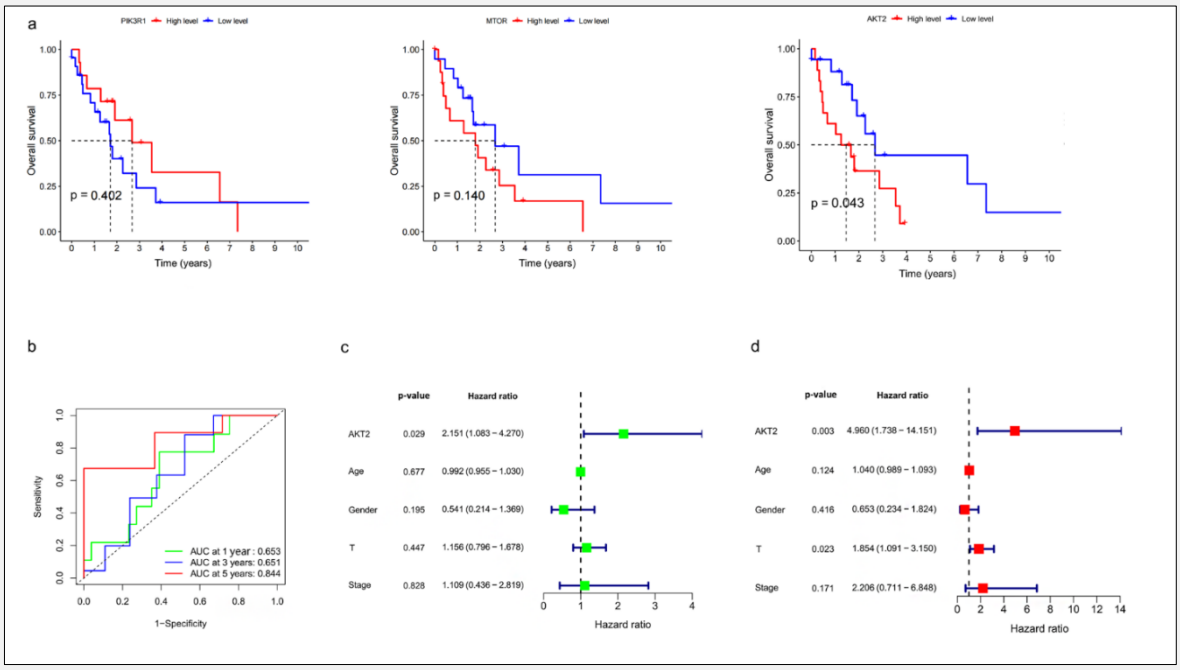

Figure 3. Survival analysis of patients with high/low key gene expression.

a) Kaplan–Meier curves of OS in PI3KR1, mTOR, and AKT2 high/low expression groups.
b) ROC curve of 5-year survival prediction based on AKT2 for LUAD.
c) Univariate independent prognostic correlation analyses with high/low AKT2 expression.
d) Multivariate independent prognostic correlation analyses with high/low AKT2 expression.

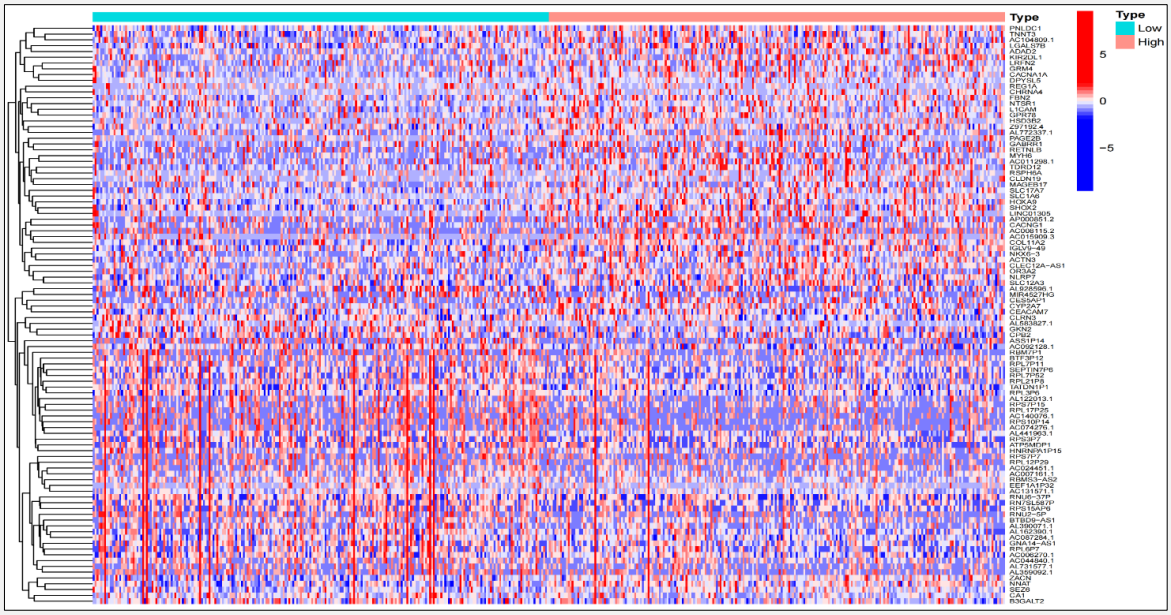

Figure 4. Heatmap of co-expression genes analysis with AKT2.

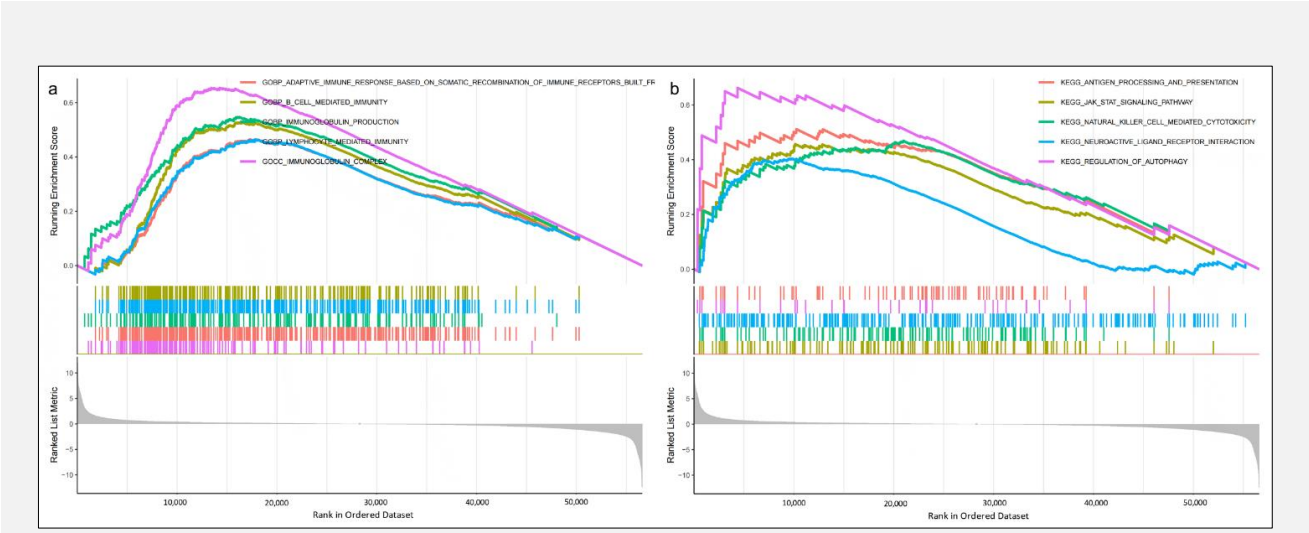

Figure 5. GSEA analysis in the AKT2 high expression LUAD patients.

a) GO analysis results.
b) KEGG analysis results.

were negatively regulated by AKT2 expression, and 203 genes were positively regulated.

GO, KEGG, and GSEA analysis results

Based on the AKT2 level in tumor tissue samples, the tumor tissue samples were divided into two groups: high expression group and low expression group, and GSEA analysis was conducted. GSEA analysis revealed that in the AKT2 high expression group, various pathways and processes related to immune reaction were significantly enriched. Specifically, GOCC_immunoglobulin complex, GOBP_adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin super family domains, GOBP_immunoglobulin production, GOBP_lymphocyte mediated immunity, and GOBP_cell mediated immunity exhibited increased activity (Figure 5a). Furthermore, enrichment was observed in pathways such as KEGG_JAK STAT signaling pathway, KEGG_natural killer cell mediated cytotoxicity, KEGG_neuroactive ligand receptor interaction, KEGG_regulation of autophagy, and KEGG_antigen processing and presentation pathway (Figure 5b).

Correlation between AKT2 expression and differential infiltration of γδT cells and Tregs cells in the tumor microenvironment

An analysis was conducted comparing immune cell infiltration across different AKT2 expression statuses (Figure 6a), and the correlation between varying levels of AKT2 expression and immune cell infiltration within tumor tissues was investigated (Figure 6b). The results revealed that tumor tissues characterized by high expression of AKT2 tended to have higher levels of follicular helper T cells, regulatory T cells (Tregs), macrophages M1, $CD8^+$ T cells, macrophages M0, and resting natural killer (NK) cells and lower levels of resting dendritic cells, resting mast cells, activated dendritic cells, γδT cells, monocytes, resting memory $CD4^+$ T cells, macrophages M2, and memory B cells (Figure 6c). The results indicate that in patients with high AKT2 expression, the infiltration of Tregs cells that leads to tumor immunosuppression was increased and cells such as γδT cells, which are conducive to tumor immune enhancement and cytotoxicity capabilities, were significantly reduced. There may be significant immune evasion in patients with LUAD and associated high AKT2 expression.

High expression of AKT2 may be positively correlated with the response efficacy of immune checkpoint inhibitors (ICIs)

The correlation between AKT2 expression and the expression of common ICIs was analyzed. The results revealed that there was a positive regulatory correlation between the expression of AKT2 and the expression of multiple common ICIs. The immune checkpoints with the highest correlation coefficients were CD276 (B7H3), lymphocyte activation gene-3 (LAG3), PDCD1 (PD-1), and TNFRSF4 (OX40), all of which demonstrated coefficients with values higher than 0.25 (Figure 7a). High expression of AKT2 was also significantly and positively correlated with high TMB, with a correlation coefficient value of 0.18 (Figure 7b).

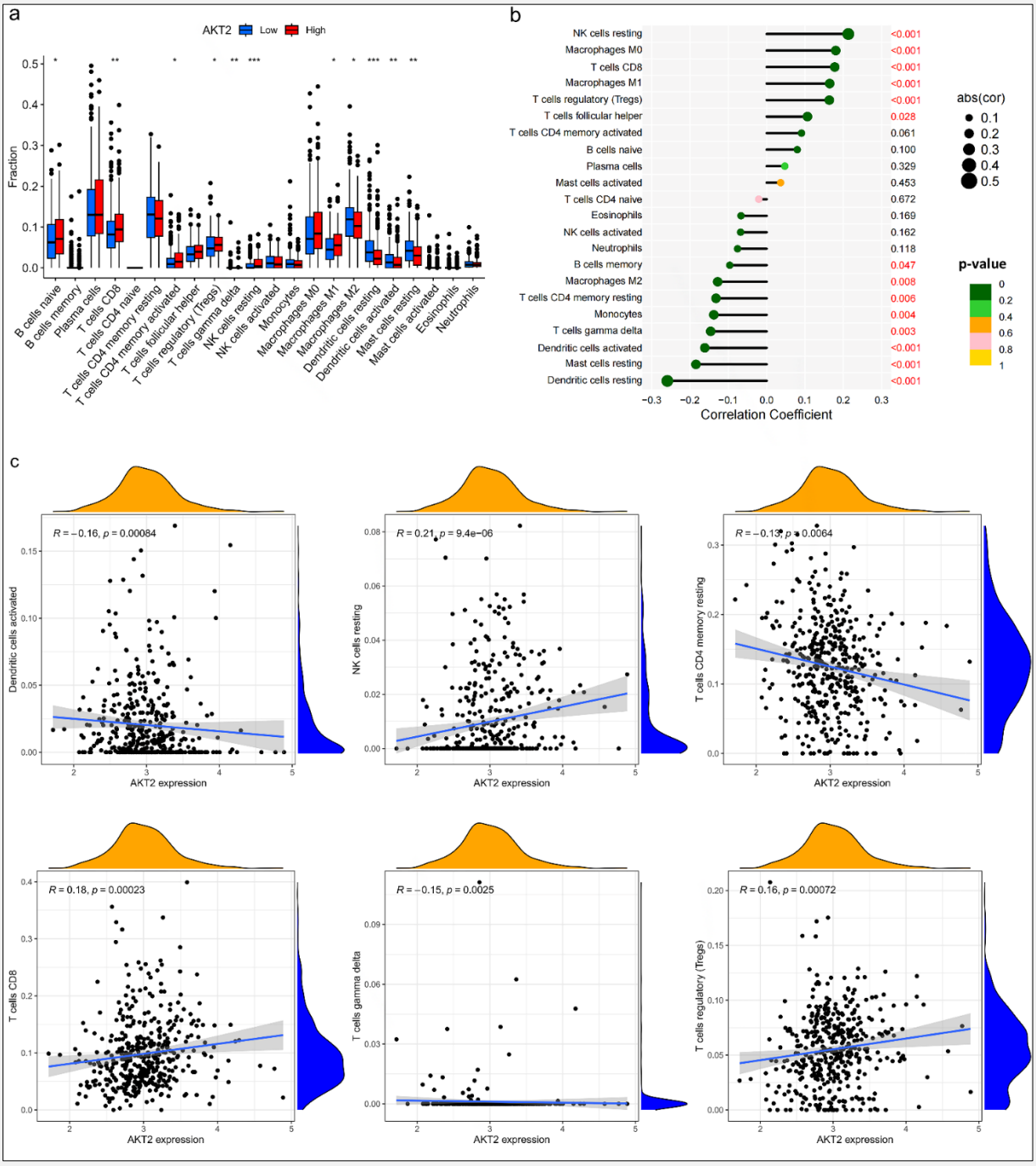

Figure 6. Correlation analysis of high/low expression of AKT2, immune cell infiltration in tumor tissues, and infiltration of immune cell subsets (* p < 0.05, ** p < 0.01, *** p < 0.001).

a) Comparing immune cell infiltration across different AKT2 expression statuses.
b) The correlation between varying levels of AKT2 expression and immune cell infiltration within tumor tissues.
c) Infiltration of activated dendritic cells, resting NK cells, $CD4^+$ memory resting T cells, $CD8^+$ T cells, γδT, and Tregs in tumor tissues.

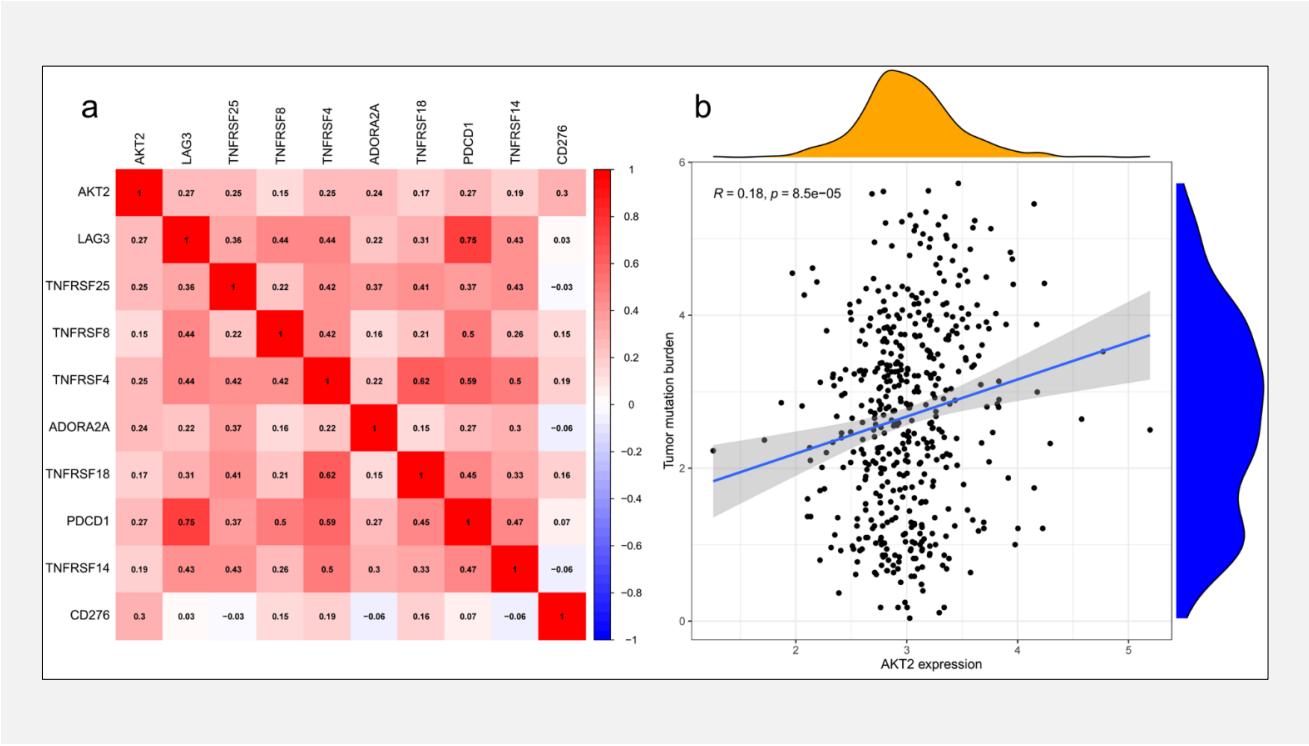

Figure 7. Correlation gene expression analysis of AKT2 with ICIs and TMB.

a) Correlation between AKT2 and common immune checkpoint expression, b) Correlation between AKT2 expression and TMB.

DISCUSSION

Patients undergoing treatment with ICIs are typically screened using biomarkers, including PD-L1 expression levels on tumor cell surfaces, TMB, or MSI-H/dMMR. Patients identified through the aforementioned markers exhibit enhanced survival benefits in both monotherapy and combination therapy [6]. Conversely, patients with NSCLC possessing driver gene mutations such as epidermal growth factor receptor (EGFR), ALT, c-ros oncogene 1 receptor kinase (ROS-1), and cellular-mesenchymal to epithelial transition factor (c-MET) show poorer responses to ICIs. For instance, patients with NSCLC harboring EGFR mutations typically exhibit reduced efficacy to PD-1/L1 antibody treatments [7], and the concurrent administration of immune therapy with targeted therapy precipitates an escalation in immune related toxicities such as immune related-pneumonia and rapid disease progression [8]. Given the limited efficacy of ICI monotherapy, there is ongoing research to develop more specific biomarkers or evaluation systems to identify patient cohorts likely to benefit more significantly. Meanwhile, another way is exploring the targets that may further enhance the efficacy of immunotherapy. Nevertheless, efforts to identify biomarkers with high selectivity or targets with potential to improve the efficacy beyond the existing ones have not yet been successful [9].

In the past, the investigation of these biomarkers or targets was primarily focused on tumor cells, such as TMB, MSI-H/dMMR, among others. The combined positive score (CPS), which incorporates the PD-L1 expression level of TILs, is utilized in the assessment of PD-L1 expression; however, its application is restricted to specific tumor types such as esophageal cancer and cervical cancer. In the context of NSCLC, PD-L1 expression on the surface of tumor cells is the sole indicator used, without an evaluation of the immune microenvironment. Extensive research has been conducted on the use of indicators such as TILs to aid in the prediction of the response efficacy of ICIs [10]; nevertheless, their potential as biomarkers for immunotherapy still requires further evaluation.

The expression level of PD-L1 protein in tumor cells was evaluated using the immune histochemistry (IHC) method, which depends on the availability of pathological sections for detection. To obtain more accurate results, it requires a minimum of 100 stained tumor cells within the sections and has higher requirements for tissue samples. For patients with advanced NSCLC, procedures such as CT-guided percutaneous lung puncture or flexible bronchoscopic lung biopsy are mostly used to obtain tumor tissue for detection. However, the obtained tissue volume is often limited or exhibits incomplete tissue morphology, precluding PD-L1 detection. Comparatively, high-throughput sequencing (NGS)

technology has lower requirements on the sample, and is increasingly utilized for gene sequencing, particularly with widespread application in targeted therapies, yielding comprehensive insights into genetic profiles of patients. Therefore, it is more convenient and feasible to identify the biomarker or target that is related to the efficacy of ICIs based on the gene mutation status of patients. This can enable clinicians to predict the efficacy of immunotherapy more easily and quickly, which is of great clinical significance in guiding the formulation of personalized treatment plans. Meanwhile, it can also guide the researcher in the development of new drugs targeting these genes or of proteins that could enhance the efficacy of immunotherapy.

Based on the abovementioned considerations, an investigation was conducted to explore the relationship of gene expression and potential efficacy of ICIs from the perspective of tumor microenvironment by analyzing the LUAD genome in the TCGA database and assessing the differences in the distribution of infiltrating lymphocytes across various levels of different target gene expression levels in the tumor immune microenvironment. According to the findings of this study, the expression of AKT2 in tumor cells was significantly upregulated as compared with that in the precancerous normal tissues. The difference in this expression was not correlated with age, gender, and tumor stage of the patients, indicating a significant correlation between increased AKT2 expression and LUAD onset and progression. The OS analyses of the high and low AKT2 expression groups in this study also confirmed that high AKT2 expression can lead to shorter expected survival period.

In this study, enrichment analysis of genes that were significantly expressed in tumor tissues compared with normal tissues was conducted; the co-expressed genes were highly clustered in the PI3K-AKT-mTOR pathway, which is consistent with the results of a previous study [11]. GSEA analysis can detect the effect of a certain gene expression on subtle but coordinated changes in biological pathways; hence, it is more sensitive and accurate in terms of biological processes. The analysis revealed that the high expression of AKT2 had significant effects on both innate and adaptive immunity. This includes effects on NK cell-mediated cytotoxicity, antigen processing and presentation, and antibody-mediated adaptive immune response, in addition to a more significant effect on the regulation of cellular autophagy. This finding suggests that the high expression of AKT2 has a significant impact on the dysregulation of tumor immune response-related functions and pathways.

Furthermore, the analysis of immune cell aggregation in the tumor microenvironment revealed a higher concentration of $CD8^+$ T cells, follicular helper T cells, and M1 macrophages in the AKT2 high expression group than in the AKT2 low expression group, indicative of an anti-tumor immune response in the AKT2 high expression group. However, the infiltration of $CD4^+$ T cells and activated dendritic cells decreased in the high expression group, whereas the presence of Tregs cells and resting NK cells in tumor immunosuppression increased. This suggests that the activation of innate immunity and effector T cells in the high expression group were suppressed, which was not conducive to the continuous amplification of tumor immune response. Specifically, a significant reduction in γδT cells conducive for tumor immune surveillance and cytotoxicity was observed, suggesting that there may be more significant immune evasion in LUAD with high expression of AKT2. With this finding, drugs targeting AKT2 may help transform "cold tumors" into "hot tumors" by modifying the tumor immune microenvironment and thus improving the efficacy of immunotherapy.

T cells are classified into two major types of cells: αβ T cells and γδT cells. While αβ T cells are widely used in CAR-T therapy, γδT cells only account for 1% to 5% of peripheral blood T cells. γδT cells have the ability to directly recognize tumor-associated antigens and are not limited by major histocompatibility complexes (MHCs). Moreover, they can be activated in the early stages of tumorigenesis, even when tumor burden is minimal, which is pivotal for tumor immune monitoring [12].

γδT cells exert a positive effect on anti-tumor immune response. Studies have revealed a significant correlation between the number of γδT cells in breast tissue and the survival duration of patients with breast cancer, indicating that a higher count of these cells corresponds to longer survival times [13]. Similarly, research in hepatocellular carcinoma (HCC) found substantial infiltration of γδT cells, which possess the capacity for human leukocyte antigen (HLA)-independent tumor responsiveness. The presence of γδT cells in HCC was also positively correlated with patient survival [14]. Vδ1T cells, a subtype of γδT cells, have been detected in lung tumors and non-cancerous lung tissues. The presence of Vδ1 T cells in lung tumors has been associated with the maintenance of sustained remission post-surgery [15]. Research has shown that β2-microglobulin deficiency leads to elevated PD-1 expression in Vδ1 and Vδ3 cells, subtypes of γδT cells. The blockade of PD-1 permits γδT cells to eradicate tumor cells without the constraints of human leukocyte antigen class 1 (HLA-1) [16]. As innate immune system effectors, γδT cells operate independently of major histocompatibility complex restrictions, making them excellent candidates for cancer immunotherapy. Currently, global research on γδT cells immunotherapy has gained momentum, primarily for the treatment of solid tumors such as lung and kidney malignancies [17]. The findings of this study reveal that LUAD with high AKT2 expression is associated with a deficiency of γδT cells in the tumor microenvironment, suggesting drugs targeting AKT2 may be a potential combination therapy for γδT cell therapy to enhance the efficacy in this context.

Furthermore, we also found a positive regulatory correlation between AKT2 and the expression of genes including PD-1, LAG3, and OX40, which are potential immune checkpoints for developing anti-tumors. Moreover, TMB is also relatively higher in patients with high

AKT2 expression, suggesting that patients with high AKT2 expression may have a potential higher response to immunotherapy. Using the expression of AKT2 as a biomarker may assist clinicians to predict the efficacy of immunotherapy and select the best response patient population.

The upregulation of AKT2 expression is associated with poor prognosis for patients with LUAD. The primary finding of this study is that the tumor microenvironment of LUAD patients in the groups with high AKT2 expression exhibited immunosuppressive characteristics such as increased Tregs cell infiltration and decreased γδT cell aggregation. Consequently, AKT2 may serve as a potential target for improving the clinical outcome of ICIs by modifying the tumor immune microenvironment, and the combination of AKT2 targeting drugs may be a promising strategy to enhance the efficacy of immunotherapy. Meanwhile, this study also suggests that in the population with LUAD, high expression of AKT2 is directly associated with poor survival prognosis of the patients.

This work advances our understanding in that high AKT2 expression may drive LUAD patients toward forming an immunosuppressive microenvironment, manifested as inhibition of both innate and adaptive immunity, along with an elevation in inhibitory TILs. These findings provide a rationale for exploring synergistic targets in immunotherapies targeting pathways such as PI3K-AKT-mTOR.

However, this study has some limitations. First, as a retrospective study, the quality of gene transcription and clinical data may inherently differ from that of prospective studies. Second, given the multifactorial nature of tumor microenvironment formation, the generalizability of our findings to more rigorously-designed prospective studies remains to be established.

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Availability of Data and Materials:
Data related to the current study are available from the corresponding author on reasonable request.

Declaration of Interest:
The authors declare that they have no competing interests.

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