Original Article

PCK2 inhibits lung adenocarcinoma tumor cell immune escape through oxidative stress-induced senescence as a potential therapeutic target

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Background: Our research aimed to better understand how phosphoenolpyruvate carboxykinase 2 (*PCK2*) is linked to survival outcomes in lung cancer patients.

Methods: We confirmed *PCK2* expression and its association with the outcome of lung cancer patients using The Cancer Genome Atlas (TCGA) database. *PCK2* and immune cell connections were investigated using data from the Tumor IMmune Estimation Resource (TIMER) and TCGA repositories. We used the CancerSEA database to examine the links between *PCK2* expression and the efficiency of lung adenocarcinomas, and a T-distributed Stochastic Neighbor Embedding (T-SNE) map was constructed to show the expression profile of *PCK2* in single cells in TCGA lung adenocarcinoma samples. The potential mechanism of action was finally investigated using Gene Set Enrichment Analysis (GSEA) enrichment analysis, Gene Ontology (GO) pathway enrichment analysis, and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis.

Results: The expression of PCK was lower in lung adenocarcinoma tumor tissues than in paracancerous tissues. Patients with lung adenocarcinoma who expressed *PCK2* at high levels fared better in overall survival (OS), disease-specific survival (DSS), and progression free interval (PFI). *PCK2* was positively correlated with programmed cell death 1 (*PDCD1*) expression, and its mutation rate in lung adenocarcinoma was 0.53%. CancerSEA research revealed that in lung adenocarcinoma, *PCK2* was negatively correlated with epithelial-mesenchymal transition (EMT) and hypoxia. Gene ontology and KEGG enrichment analysis revealed *PCK2*-coexpressed genes influenced the onset and progression of lung adenocarcinoma by modulating the activity of DNA-binding transcriptional activators, the specificity of RNA polymerase II, the interaction between neuroactive ligands and their receptors, and the cAMP signaling pathway. The prognosis for lung adenocarcinoma was shown to vary according to whether *PCK2* was involved in the response to oxidative stress-induced senescence, gene silencing, cell cycle, and other biological processes.

Conclusions: An increased expression of *PCK2* may be employed as a novel prognostic biomarker in patients with lung adenocarcinoma and has been shown to increase OS, DSS, and PFI. Improving the prognosis of lung adenocarcinoma by interference with *PCK2* may be possible since it induces senescence through the oxidative stress response and blocks the immune escape of tumor cells. These results point to a probable target anticancer treatment development in lung adenocarcinoma.

Keywords: Phosphoenolpyruvate carboxykinase 2 (PCK2); lung adenocarcinoma; immune infiltration; gluconeogenesis; prognosis

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Introduction

Non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) account for over 95% of lung cancer diagnoses and deaths globally (1). Lung adenocarcinoma is a common form of lung cancer, accounting for roughly 40% of all lung cancer cases (2). NSCLC is further subdivided into three types: squamous cell carcinoma, adenocarcinoma, and large cell carcinoma (2). Surgery followed by radiation treatment and chemotherapy has been shown to considerably increase the 5-year survival rate for patients with early-stage lung adenocarcinoma (with stages IA, IB, IIA, and IIB as 83%, 68%, 60%, and 53%, respectively) (3). On the other hand, individuals with advanced lung adenocarcinoma are seldom treated with the same methods, and their 5-year survival rate is approximately 10% (4). While historically, patients with lung adenocarcinoma were treated with minimally invasive surgery, radiotherapy, and chemotherapy, with the advent and widespread use of biological therapy and immunotherapy, these modalities have been largely replaced, and patient survival times have gradually increased (5). The postoperative recurrence rate and the death rate from lung cancer may be drastically decreased in patients who undergo urgent surgical therapy (6). As a result, it is crucial that we find effective target molecules in both early diagnosis and therapy as promptly as possible.

At present, the abnormal metabolism of tumors is a hot research topic, and most studies are conducted on the three basic metabolisms (7). Gluconeogenesis refers to the process in which cells use non-sugar precursors, such as glycerol, lactic acid, pyruvic acid, and sugar-producing amino acids, to generate free glucose or glycogen (8). In many tumor cells, gluconeogenesis plays a crucial regulatory function in the aerobic glycolysis metabolic pathway, although this mechanism receives far less attention than glycolysis and oxidative phosphorylation in cancer research (9,10). Despite the availability of oxygen, glycolysis continues to be an important energy source for many tumor cells, and the term "aerobic glycolysis of tumors" (11) describes this phenomenon. The gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PEPCK) catalysis the GTP-dependent oxaloacetate (OAA) to phosphoenolpyruvate (PEP) reaction. The two versions of this enzyme are PCK1 (PEPCK-C) in the cytoplasm and phosphoenolpyruvate carboxykinase 2 (*PCK2*) (PEPCK-M) in the mitochondria (12). Recent research has shown the significance of *PCK2* in biological processes including the emergence and progression of many cancers (13). Renal cell carcinoma development may be slowed by increasing endoplasmic reticulum stress and the responsiveness to the drug sunitinib when genes normally silenced by epigenetic mechanisms are activated. Transcriptional activation of *PCK2* by PGC-1β and ERR-α promotes glutamine metabolism and colorectal cancer survival (14), while down-regulation of *PCK2* in melanoma tumor-regenerating cells modifies the tricarboxylic acid cycle (15). However, research on the function of *PCK2* in lung cancer is limited.

In this study, we primarily analysed several datasets, including The Cancer Genome Atlas (TCGA), to determine how often *PCK2* was expressed and how well it predicted survival for patients with lung adenocarcinoma. To further investigate the impact of *PCK2* on tumor cell behavior and the underlying mechanism, we conducted bioinformatics analysis and validation. We present this article in accordance with the TRIPOD reporting checklist (available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-542/rc).

Highlight box

Key findings

- An increased expression of *PCK2* may be employed as a novel prognostic biomarker in patients with lung adenocarcinoma and has been shown to increase OS, DSS, and PFI.

What is known and what is new?

- Transcriptional activation of *PCK2* by PGC-1β and ERR-α promotes glutamine metabolism and colorectal cancer survival.
- This study investigated the impact of *PCK2* on tumor cell behavior and the underlying mechanism.

What is the implication, and what should change now?

- Improving the prognosis of lung adenocarcinoma by interference with *PCK2* may be possible since it induces senescence through the oxidative stress response and blocks the immune escape of tumor cells. These results point to a probable target anticancer treatment development in lung adenocarcinoma.

Methods

Methods for data mining in the TCGA database

We investigated *PCK2* expression in TCGA pan-cancer samples and in lung cancer and adjacent tissues.

PCK2 and lung cancer TCGA database clinical parameters

Lung cancer patient clinical parameters were obtained

from the TCGA database, and the link between *PCK2* and these clinical indicators and prognosis was assessed. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Creating nomograms and assessing their effectiveness

Nomograms were constructed using data from the multivariate analysis. The predictive ability of the nomogram was assessed with the use of a calibration curve.

Protein-gene interaction network diagram building using the GeneMania and STRING databases

The GeneMania database is used to generate assumptions about gene function, analyze gene lists, and prioritize genes for functional analysis. Given a list of query genes, GeneMania uses a large number of genomics and proteomics data to find genes with similar functions. Another use of GeneMania is for predicting gene function. Given a query gene, GeneMania will identify genes that may share functions with it based on their interactions. String database (https://string-db.org/). It is a database that searches for interactions between proteins. This includes both direct physical interactions between proteins and indirect functional correlations between proteins. Using GeneMania, we mapped out the genetic relationships between *PCK2* genes and modified those in close proximity. The *PCK2* protein-protein interaction (PPI) network was also generated using the STRING database for comparison.

Genome-wide analysis of PCK2 gene co-expression

Using data mining on the TCGA database, we determined which genes were positively or negatively co-expressed with *PCK2*.

PCK2 and immune cells: a link between the TIMER and TCGA databases

To visualize the association between *PCK2* expression and different immune cells in lung adenocarcinoma, we built a stick figure through the TIMER database (https://cistrome.shinyapps.io/timer/).

Examining mutations in genes

Change frequency, mutation site information, a mutation type, and a 3D structure of all TCGA cancer candidate proteins were analysed in the cBioPortal database (including lung adenocarcinoma).

Sequencing of individual cells

CancerSEA is a specialized database for single-cell sequencing that may reveal a variety of functional states of cancer cells at the single-cell level. Single-cell sequencing data was used to study *PCK2* expression and the performance of lung adenocarcinomas correlation. T-distributed Stochastic Neighbor Embedding (T-SNE) diagram shows the expression profile of *PCK2* in single cells of TCGA samples.

Analyzing the UALCAN database to compare promoter methylation levels across lung cancer patients

To examine and contrast the promoter methylation levels of various lung adenocarcinoma patients, their information was entered into the UALCAN database for CPTAC analysis.

Gene set enrichment analysis (GSEA)

The biological role of *PCK2* in lung cancer was investigated using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. A potent bioinformatics method, GO analysis may be used to identify *PCK2*-related cellular components (CCs), molecular activities (MFs), and biological processes (BPs). The fundamental mechanism of *PCK2* was investigated using GSEA (16).

Statistical analysis

Web resources were used to automatically compute the statistical analyses. Statistical significance was assumed at a P value or a log rank P value of <0.05.

Results

Downregulation of PCK2 expression in lung adenocarcinoma

Pan-cancer analysis using the TCGA database revealed the expression of *PCK2* was reduced in most tumors (*Figure 1A*). In the paired and unpaired tumor tissues of lung adenocarcinoma, paracancerous tissues had a greater *PCK2* expression than cancerous ones (*Figure 1B,1C*), and

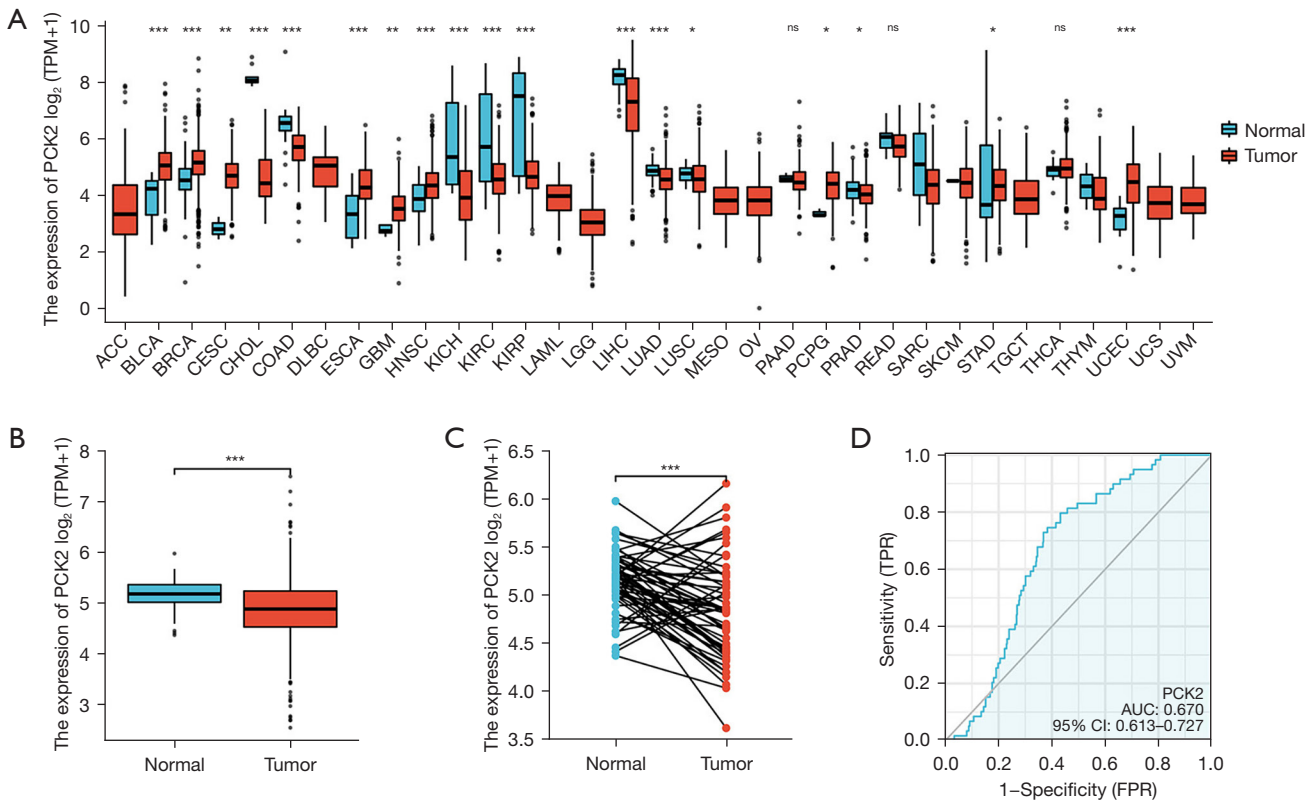

Figure 1 *PCK2* expression is downregulated in lung adenocarcinomas. (A) Expression level of *PCK2* in pan-cancer; (B) expression level of *PCK2* in adjacent tissues (n=59) and unpaired lung adenocarcinoma tissues (n=535) in the TCGA database; (C) expression levels of *PCK2* in paired lung adenocarcinoma tissues (n=57) and paracancerous tissues (n=57) in the TCGA database; (D) ROC curve analysis. The difference is statistically significant when *P<0.05, **P<0.01, and ***P<0.001. PCK2, phosphoenolpyruvate carboxykinase 2; TPM, transcripts per million; TPR, true positive rate; ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, brain lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma ; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma; FPR, false positive rate; ns, no statistical significance; TCGA, The Cancer Genome Atlas; ROC, receiver operator characteristic; AUC, area under the curve.

the area under the curve (AUC) of the receiver operator characteristic (ROC) was 0.670. This result indicates *PCK2* also has certain predictive research value (*Figure 1D*).

PCK2 and lung cancer prognosis in TCGA database

In the TCGA database, we discovered that patients with high expressions of *PCK2* in their lung adenocarcinoma had superior overall survival (OS), disease-specific survival (DSS), and progression-free interval (PFI) (*Figure 2*).

PCK2 expression in TCGA and lung cancer clinical data

Patients with reduced expression of *PCK2* had a greater T

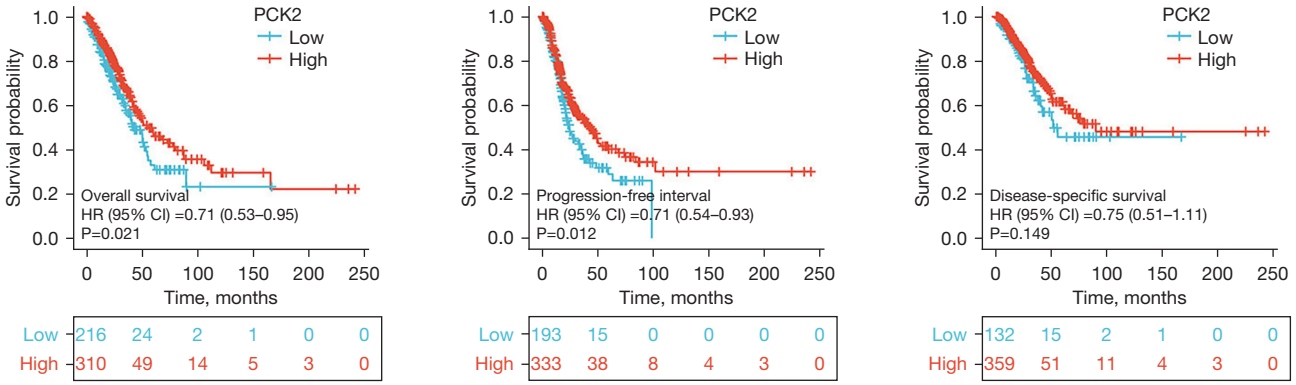

Figure 2 Relationship between the prognosis of lung adenocarcinoma patients and *PCK2* expression in the TCGA database. PCK2, phosphoenolpyruvate carboxykinase 2; TCGA, The Cancer Genome Atlas; HR, hazard ratio.

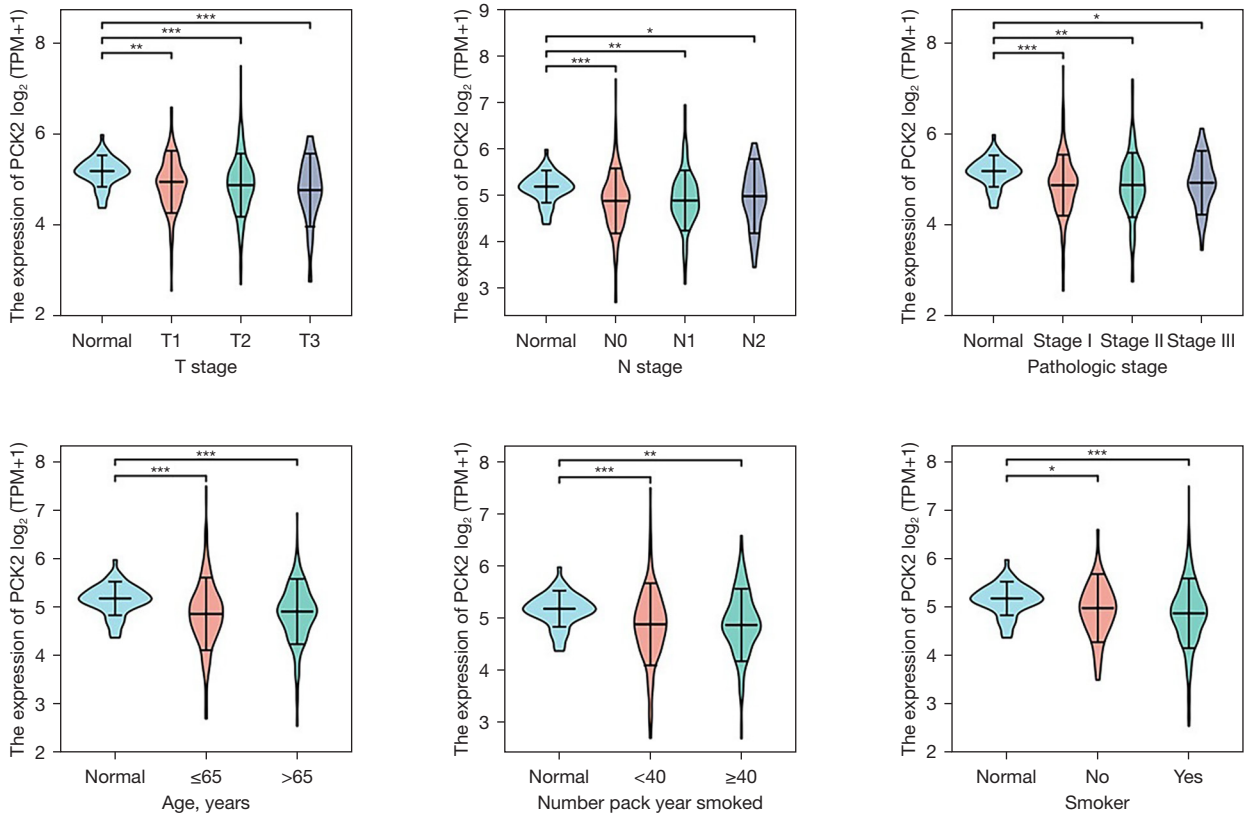

Figure 3 The relationship between patients with the clinical data of lung adenocarcinoma and *PCK2* expression. ***P<0.001, **P<0.01, *P<0.05. PCK2, phosphoenolpyruvate carboxykinase 2; TPM, transcripts per million.

stage, N stage, pathological stage, and pathological grade when compared to the clinical data of patients with lung adenocarcinoma, which is consistent with the expression of prior study findings (*Figure 3*).

Nomogram construction

Using multivariate analytic findings, we built a nomogram from the TCGA database to predict survival rates for

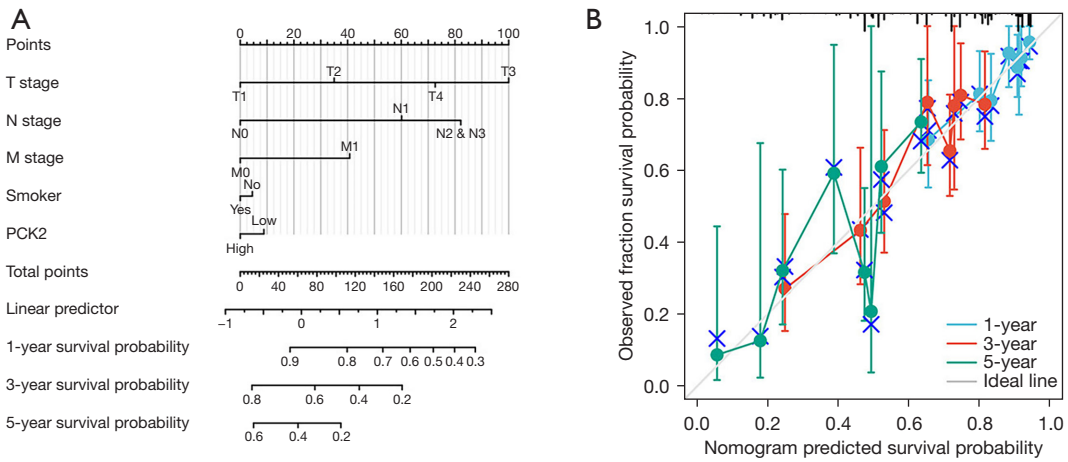

Figure 4 The nomogram and calibration plot for lung cancer patients. (A) Nomogram for 1-, 3-, and 5-year survival prediction in lung cancer patients; (B) calibration plot of the nomogram for OS likelihood prediction. PCK2, phosphoenolpyruvate carboxykinase 2; OS, overall survival.

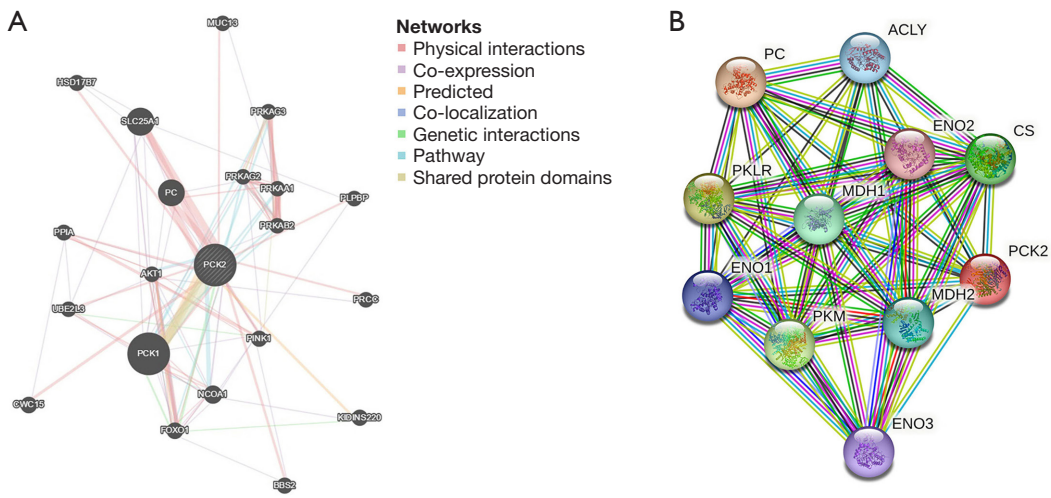

Figure 5 Identification of *PCK2* interacting genes and proteins. (A) Gene-gene interaction network of *PCK2* in the GeneMania database; (B) PPI network of *PCK2* in the STRING database. PCK2, phosphoenolpyruvate carboxykinase 2; PPI, protein-protein interaction.

1-, 3-, and 5-year in patients with lung adenocarcinoma. The C-index of the nomogram was 0.683 (0.658–0.707) (*Figure 4A*), and the calibration curve demonstrated it had a predictive value (*Figure 4B*).

Genes and proteins interacting with PCK2 were identified

Using GeneMania, we formed a *PCK2* gene-gene interaction network and modified neighboring genes (*Figure 5A*). To create the PPI network for *PCK2*, we queried the STRING database for protein-protein interactions (*Figure 5B*).

PCK2 co-expression gene screening

To find genes either positively or negatively linked with *PCK2*, we mined the TCGA database for information on lung cancer and compiled a list of the most 50 positively correlated (*Figure 6A*) and negatively correlated (*Figure 6B*) genes and displayed their expressions on a co-expression heat maps.

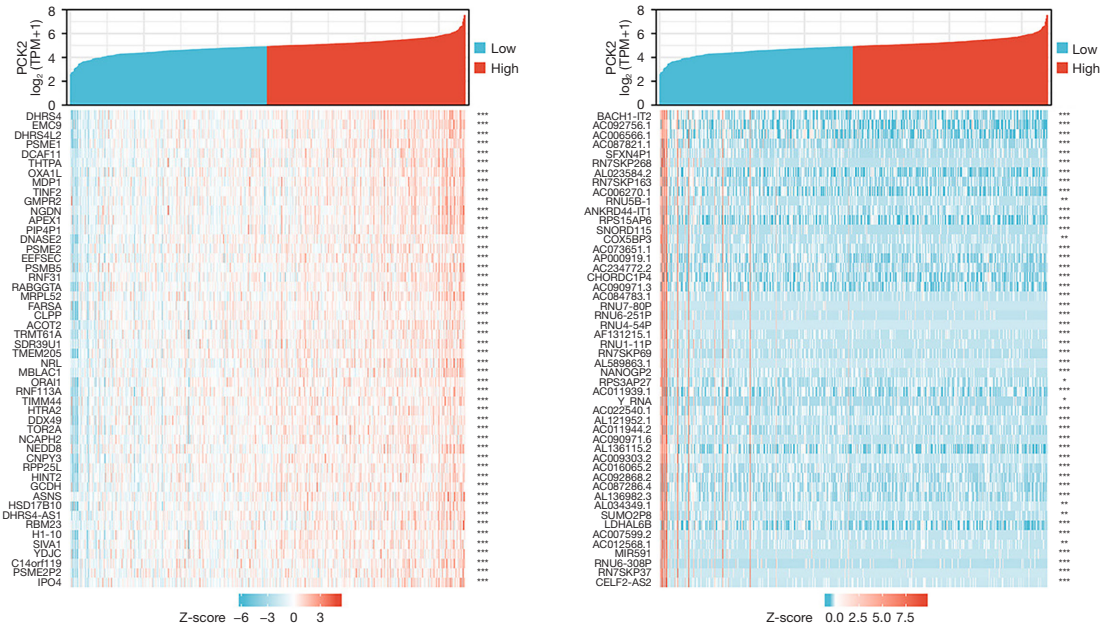

Figure 6 Heatmap of *PCK2* co-expressed genes. (A) Genes positively correlated with *PCK2* in lung adenocarcinoma; (B) genes negatively correlated with *PCK2* in lung adenocarcinoma. ***P<0.001, **P<0.01, *P<0.05. PCK2, phosphoenolpyruvate carboxykinase 2; TPM, transcripts per million.

PCK2 and immunological cells: a TIMER and TCGA database correlation

Using the TIMER database, we created a diagram depicting the *PCK2* expression and tumor purity correlation to different immune cells in lung adenocarcinoma. *PCK2* expression was correlated positively to CD4+ T cells, B cells, macrophages, CD8+ T cells, dendritic cells, and neutrophils, which are six of the eight categories of invading immune cells (*Figure 7A*). The infiltration levels of *PCK2* and aDC, Treg, iDC, TFH, DC, pDC, and Th2 cells were all positively correlated, but levels of infiltration for Tcm, T helper cells, CD8 T cells, and Th17 cells were negatively correlated. Accordingly, we performed a more in-depth analysis of the effect of *PCK2* on the TME by analyzing the correlation between *PCK2* and specific immune cells (*Figure 7B*). Additional research revealed a statistically significant positive link between *PCK2* expression and *PDCD1*, a molecule associated with immunological checkpoints, although no such difference existed between *PCK2* and *CTLA4* or *CD274* (*Figure 7C*). These results provide a foundation for further investigation and raise the possibility that *PCK2* expression is linked to immune infiltration into lung adenocarcinoma tumors. They also suggest *PCK2* plays a significant function in preventing the escape of immune by tumor cells within the lung adenocarcinoma tumor microenvironment.

Mutations of PCK2 in lung adenocarcinoma

To further understand *PCK2* gene mutation in cancer, we evaluated its various mutation statuses using the cBioPortal platform and TCGA data. In lung adenocarcinoma, the mutation rate of *PCK2* was 0.53%, while the amplification rate was 1.77% and the deep deletion rate was 0.35%, according to a global study of cancer (*Figure 8A*). *Figure 8B* shows missenses and truncations were the main mutation types in *PCK2*, and *Figure 8C* shows the change at A477T/S in the 3D structure of the PCK2 protein.

PCK2 promoter methylation in lung adenocarcinoma subtypes

Cancer and development may both be affected by promoter DNA methylation, which has been related to transcriptional suppression. In a stratified examination of lung adenocarcinoma patients, we found that *PCK2* promoter methylation decreased with disease progression, and regardless of whether they had the TP53 mutation, patients fell into a wide range of age groups, N stages, and

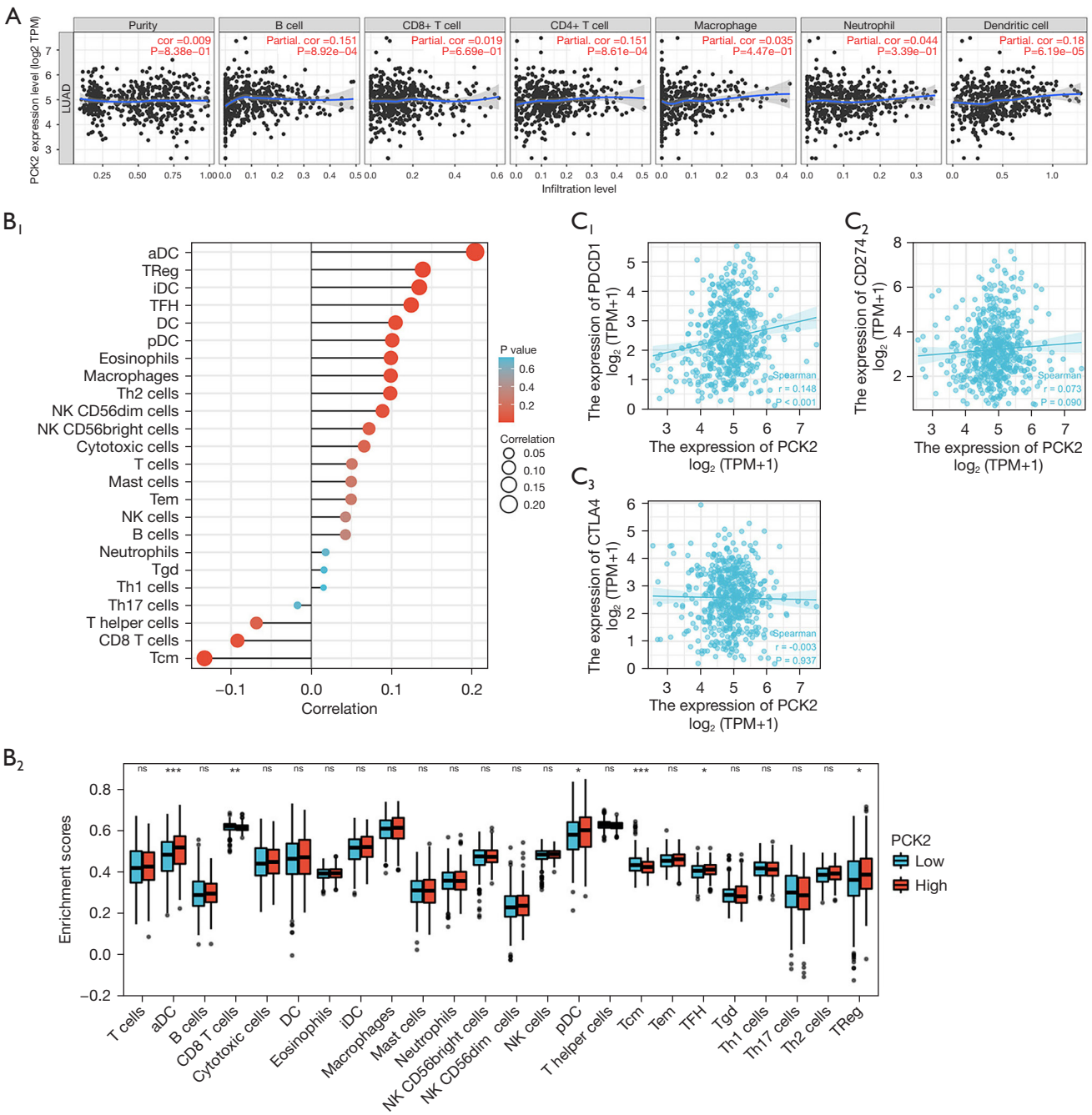

Figure 7 Relationship between *PCK2* expression and degree of immune infiltration. (A) In the TIMER database, *PCK2* may be associated with different degrees of immune cell infiltration in lung cancer. (B) In the TCGA database, *PCK2* expression may be correlated with various levels of immune cell infiltration in lung adenocarcinoma. (C) The correlation between *PCK2* expression and CTLA4, CD274, and PDCD1 is shown in a scatter plot. ***P<0.001, **P<0.01, *P<0.05, the difference is statistically significant. PCK2, phosphoenolpyruvate carboxykinase 2; TPM, transcripts per million; TCGA, The Cancer Genome Atlas; ns, no statistical significance; aDC, activated dendritic cells; iDC, interdigitating dendritic cells; NK, natural killer cell; pDC, plasma cell like dendritic cells; CTLA4, cytotoxic T-lymphocyte associated protein 4; PDCD1, programmed cell death 1.

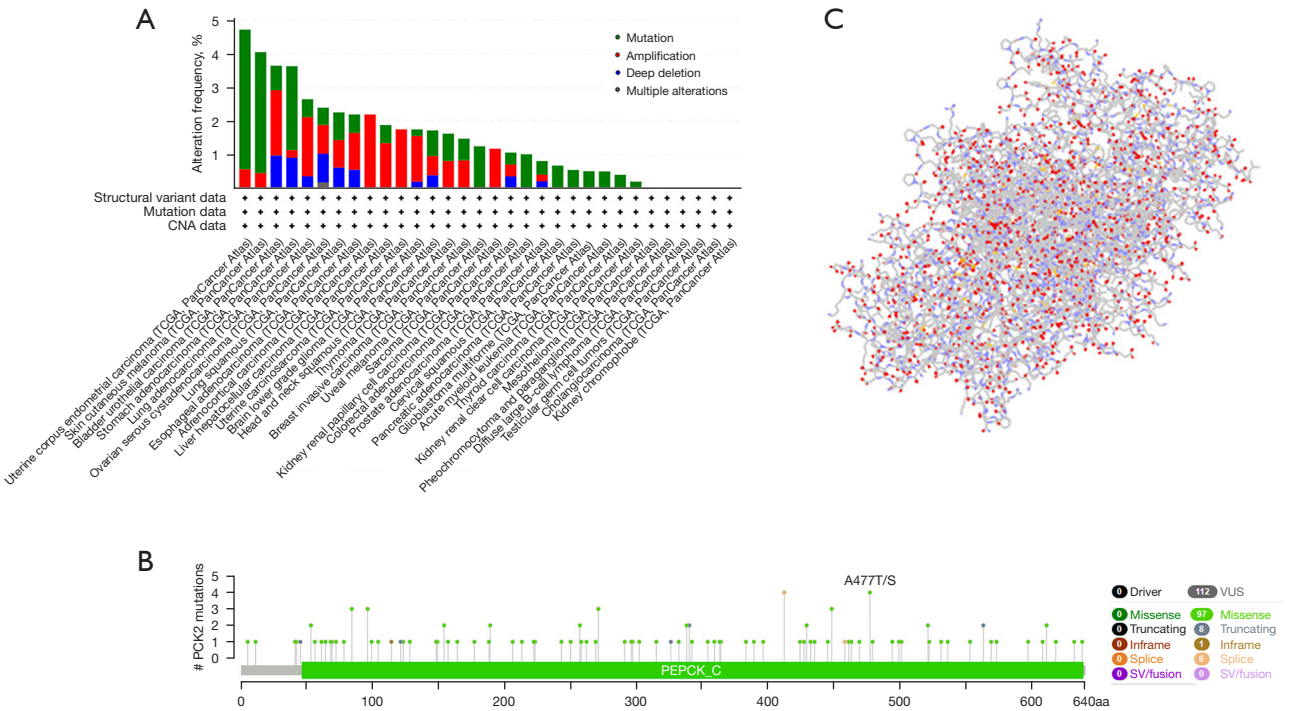

Figure 8 Lung cancer with *PCK2* gene alterations. (A) The cBioPortal displays various *PCK2* mutation types in various malignancies, including lung adenocarcinoma; (B) the cBioPortal displays various *PCK2* mutation frequencies in various tumours, including lung adenocarcinoma; (C) the 3D structure of the A477T/S mutation site in the PCK2 protein. PCK2, phosphoenolpyruvate carboxykinase 2; TCGA, The Cancer Genome Atlas; CNA, copy-number alterations; VUS, uncertain significance; SV/Fusion, structure variations/fusion.

PCK2 promoter methylation patterns. Therefore, we can speculate that the expression of transcriptional *PCK2* may be due to changes in promoter methylation (*Figure 9*).

Single-cell analysis of PCK2 expression in lung adenocarcinoma

One of the most useful tools for assessing the possible roles of candidate molecules in a single cell is single-cell transcriptome sequencing. To start, we examined the correlation between *PCK2* expression and phenotypes in a variety of tumor types using the CancerSEA database (including lung adenocarcinoma). *Figure 10A* shows that in lung cancer, *PCK2* is negatively correlated with epithelial-mesenchymal transition (EMT) and hypoxia, and has other relationships with autophagy, cell cycle, differentiation, and inflammation (*Figure 10B*). By using T-SNE plots, we also displayed the *PCK2* expression profile at the single-cell level in lung cancer (*Figure 10C*).

GO and KEGG enrichment analysis

To evaluate the role of *PCK2* in the development and progression of lung adenocarcinoma, we next performed a functional enrichment analysis. Gene ontology (GO) and KEGG enrichment analysis (*Tables 1,2*) showed genes co-expressed with *PCK2* affect DNA-binding transcriptional activator activity, RNA polymerase II specificity, neuropeptide hormone activity, neuroactive ligand-receptor interaction, dopaminergic synapse, cAMP signaling pathway, and other features involved in the occurrence and development of lung adenocarcinoma (*Figure 11*).

GSEA-based pathway prediction in signal transduction

To analyze the functionality of genes, we used Metascape to perform KEGG functional analysis online. This revealed *PCK2* may have an impact on lung cancer development by taking part in the reaction to oxidative stress-induced

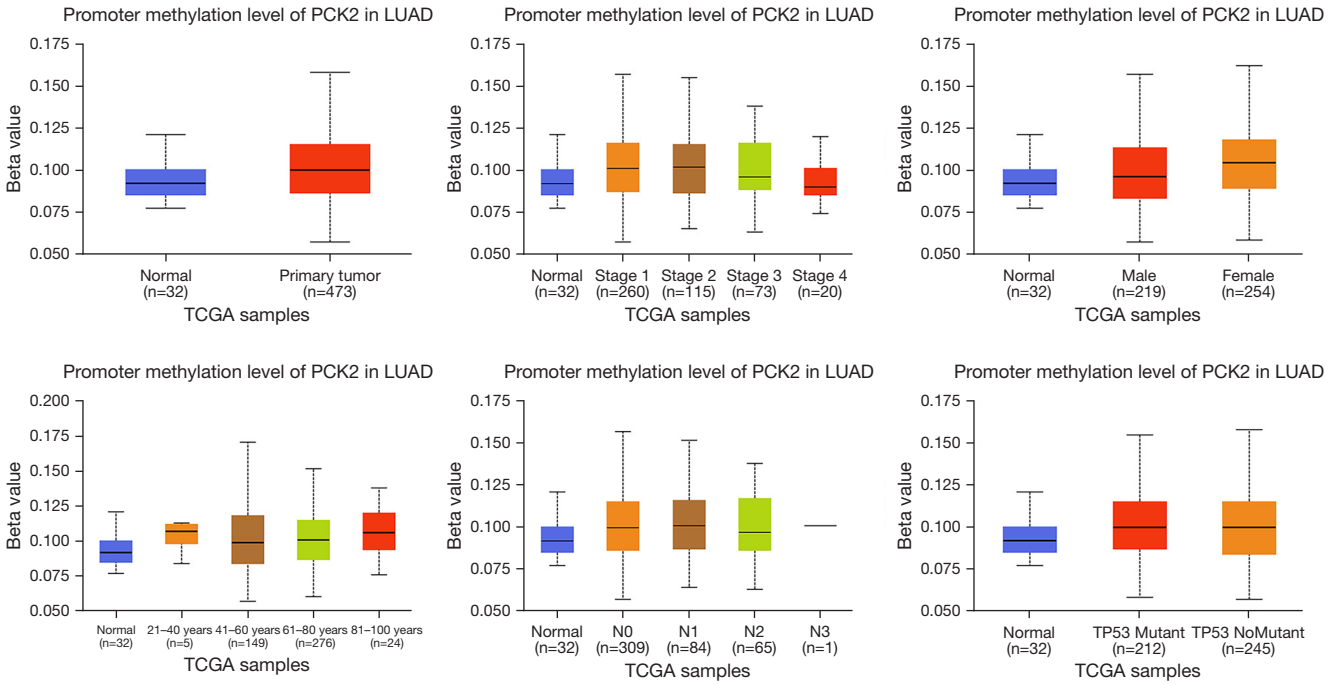

Figure 9 Promoter methylation of *PCK2* in different lung adenocarcinoma types. PCK2, phosphoenolpyruvate carboxykinase 2; LUAD, lung adenocarcinoma; TCGA, The Cancer Genome Atlas.

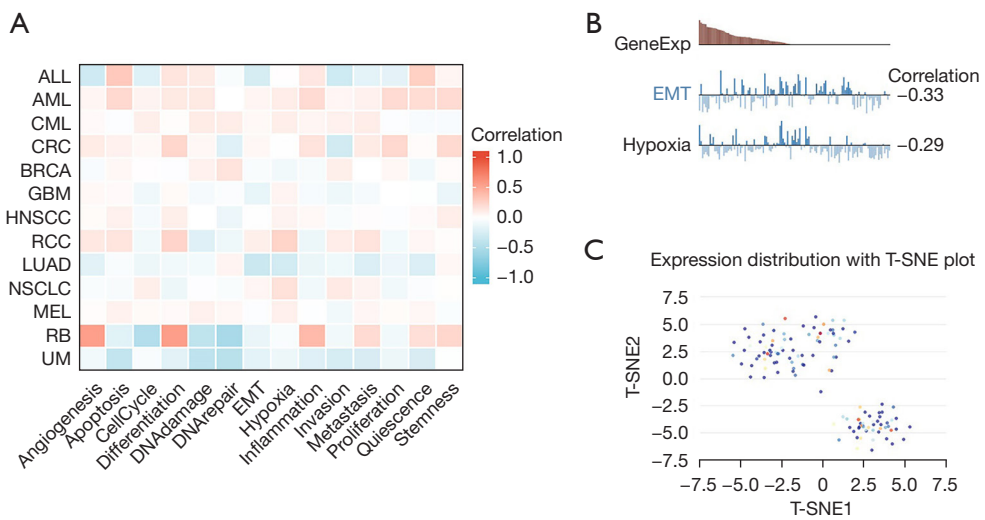

Figure 10 *PCK2* expression levels in individual cells. (A) The CancerSEA tool investigated the relationship between *PCK2* expression and various functional states in various tumors; (B) high correlation between *PCK2* expression and functional states in lung adenocarcinoma; and (C) expression profile of *PCK2* at the single-cell level in lung adenocarcinoma as shown by the T-SNE map. PCK2, phosphoenolpyruvate carboxykinase 2; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CML, chronic myelogenous leukemia; CRC, colorectal cancer; BRCA, breast invasive carcinoma; GBM, glioblastoma multiforme; HNSCC, head and neck squamous cell carcinoma; RCC, renal cell carcinoma; LUAD, lung adenocarcinoma; NSCLC, non-small cell lung carcinoma; MEL, mouse erythroleukemia; RB, retinoblastoma; UM, uveal melanoma; EMT, epithelial-mesenchymal transition; T-SNE, t-distributed stochastic neighbor embedding.

Table 1 Details of GO analysis of *PCK2*-related genes

Ontology	ID	Description	Gene ratio	Bg ratio	P value	Adjust P value	q value
BP	GO:0003002	Regionalization	16/144	351/18,670	1.35e-08	2.35e-05	2.15e-05
BP	GO:0007389	Pattern specification process	17/144	446/18,670	6.36e-08	5.54e-05	5.07e-05
BP	GO:0060579	Ventral spinal cord interneuron fate commitment	4/144	15/18,670	4.34e-06	0.002	0.002
BP	GO:0060581	Cell fate commitment involved in pattern specification	4/144	15/18,670	4.34e-06	0.002	0.002
CC	GO:0099061	Integral component of postsynaptic density membrane	4/153	50/19,717	6.08e-04	0.072	0.068
CC	GO:0099146	Intrinsic component of postsynaptic density membrane	4/153	53/19,717	7.60e-04	0.072	0.068
CC	GO:0032039	Integrator complex	3/153	28/19,717	0.001	0.082	0.078
CC	GO:0098839	Postsynaptic density membrane	4/153	74/19,717	0.003	0.096	0.091
MF	GO:0005179	Hormone activity	9/137	122/17,697	4.46e-07	1.15e-04	1.04e-04
MF	GO:0001228	DNA-binding transcription activator activity, RNA polymerase II-specific	11/137	439/17,697	6.21e-04	0.080	0.072
MF	GO:0005184	Neuropeptide hormone activity	3/137	28/17,697	0.001	0.080	0.072
MF	GO:0048018	Receptor ligand activity	11/137	482/17,697	0.001	0.080	0.072

GO, Gene Ontology; *PCK2*, phosphoenolpyruvate carboxykinase 2; BP, biological process; CC, cellular component; MF, molecular activity.

Table 2 Details of KEGG analysis of *PCK2*-related genes

Ontology	ID	Description	Gene ratio	Bg ratio	P value	Adjust P value	q value
KEGG	hsa04080	Neuroactive ligand-receptor interaction	13/55	341/8,076	3.14e-07	3.92e-05	3.37e-05
KEGG	hsa05031	Amphetamine addiction	4/55	69/8,076	0.001	0.061	0.052
KEGG	hsa04971	Gastric acid secretion	4/55	76/8,076	0.002	0.061	0.052
KEGG	hsa04728	Dopaminergic synapse	5/55	132/8,076	0.002	0.061	0.052
KEGG	hsa04024	cAMP signaling pathway	6/55	216/8,076	0.003	0.075	0.064

KEGG, Kyoto Encyclopedia of Genes and Genomes; *PCK2*, phosphoenolpyruvate carboxykinase 2.

senescence, gene silencing, cell cycle, reactive protein methylation of histone arginine, suppression of telomere responses, and DNA damage caused by apoptosis. This suggests that in lung adenocarcinoma, the prognosis might vary according to biological factors.

In light of the aforementioned prediction findings and the molecular properties of *PCK2*, we hypothesize it is a therapeutic target that causes senescence through the oxidative stress response and prevents the immunological escape of tumor cells. The experimental foundation established serves as a standard for further research (*Figure 12*).

Discussion

Lung cancer diagnoses numbered 228,000 in the United States in 2019 with around 160,000 deaths (17), demonstrating the disease has a high incidence, high fatality rate, and a poor prognosis. Lung adenocarcinoma is the most prevalent form of NSCLC (18,19), and determining its mechanism of incidence, invasion, and metastasis. Nowadays, with many technical breakthroughs in disciplines such as bioinformatics, molecular biology, and immunology, molecular targeted therapy, immunosuppressant therapy, and other methods have been widely used in clinical

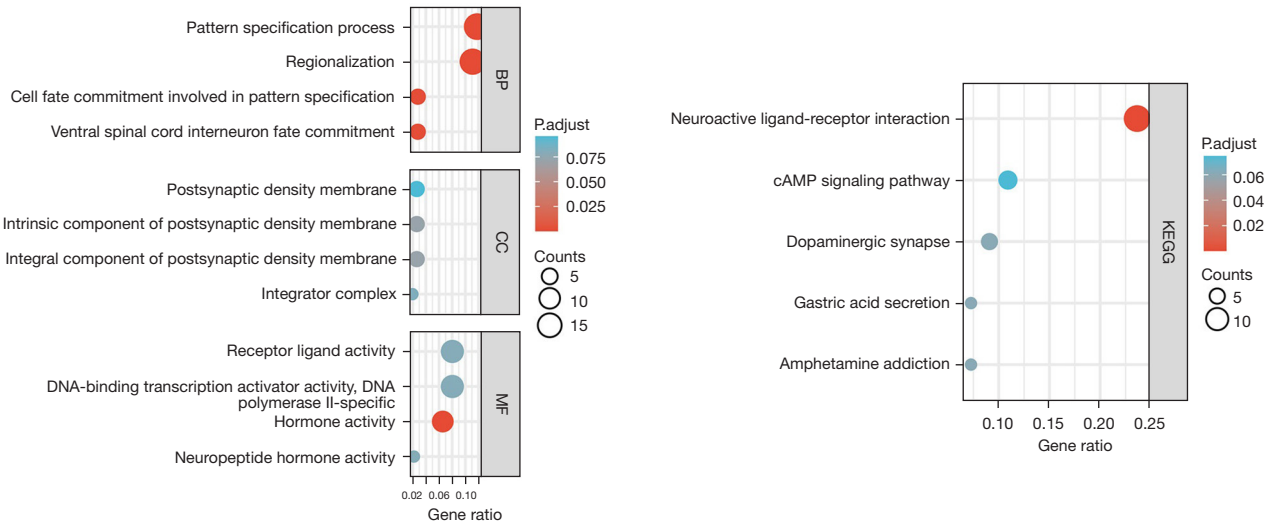

Figure 11 GO and KEGG enrichment analysis results. P<0.05, the difference is statistically significant. BP, biological process; CC, cellular component; MF, molecular function; cAMP, adenosine cyclophosphate; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

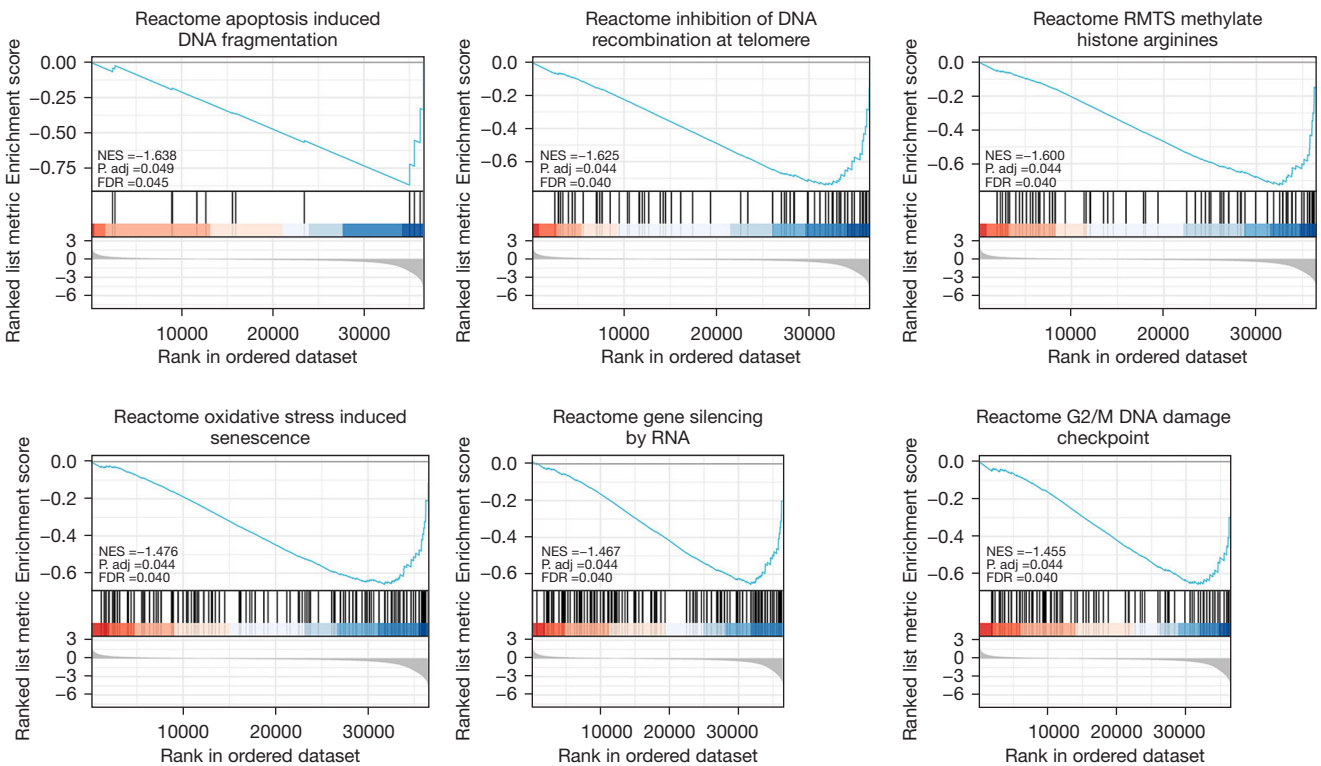

Figure 12 Six statistically significant probable relevant paths. The gene set for biological processes in ear biology from MSigDB was used. There were 1,600 different combinations of a random sample. NOM-p stands for nominal P value, NES for normalized enrichment score, and FDR-q for false discovery rate. NOM, nominal; NES, normalized corrected ES value; FDR, probability of false positive results.

practice. However, there has been no notable improvement in the survival rate for those with lung adenocarcinoma (20,21). Therefore, it is crucial to better understand the causes of the disease to identify useful biomarkers and create effective novel treatments.

Abnormal cell metabolism is a key feature of tumor development, and abnormal glucose metabolism is a basic cellular function. While targeting aerobic glycolysis in tumor cells is a promising therapeutic strategy attracting much research interest, there are fewer studies on gluconeogenesis (22). Gluconeogenesis may be thought of as the inverse of glycolysis, with its three stages mirroring the three phases of the latter. To begin, pyruvate is converted into phosphoenolpyruvate (PEP). Physiologically, the pyruvate kinase-catalyzed process in glycolysis is irreversible because its activation energy, or ΔG, is –7.5 kcal. Therefore, gluconeogenesis can only be stimulated by the presence of two high-energy links (23).

New sequencing and omics technologies have opened new avenues for researchers to learn the causes of lung adenocarcinoma and find potential treatments for the disease (24). Using bioinformatics, we hypothesized *PCK2* may play a part in the etiology of lung cancer in this investigation. Patients with high *PCK2* expression in the TCGA database had higher DSS, OS, and PFS when compared to those with a low expression. Using the database's multivariate analysis findings, we built a prediction model with a certain degree of accuracy that can potentially predict the 1-, 3-, and 5-year survival probability of lung adenocarcinoma patients. The expression of *PCK2* was shown to be positively connected with six different kinds of invading immune cells in the TIMER database, including CD4+ T cells, B cells, macrophages, neutrophils, CD8+ T cells, and dendritic cells. The PDCD1 expression was positively connected with *PCK2*, and *PCK2* in turn was positively correlated with the infiltration levels of aDC, Treg, iDC, TFH, DC, pDC, and Th2 cells. Although there is currently no clear literature suggesting experimental confirmation of *PCK2* and immune infiltration. But it may indicate that LUAD in tumor immune microenvironment is regulated by *PCK2*. It has been suggested that the tumor microenvironment of lung cancer consists of tumor associated macrophages (TAM), a small number of invasive dendritic cells and natural killer cell. Previous studies have thoroughly studied the role of tumor associated T cells in the development of lung cancer. The factors influencing the prognosis of LUAD are the activation of CD4+ Th1 cells and activated CD8+ T cells, which enhance the immune response of the body (25,26). These findings suggest *PCK2* may play a critical role in the tumor microenvironment of lung adenocarcinoma by preventing tumor cells from evading the immune system. We then examined the role of *PCK2* mutations in lung adenocarcinoma and discovered missense and truncation mutations account for most *PCK2* changes in this cancer. In addition to MNNG HOS transforming gene (c-MET) amplification, C797S mutation, and ERK pathway inhibition, previous research suggests epidermal growth factor receptor membrane/cytoplasmic/nuclear translocation may be an important cause of drug resistance in lung adenocarcinoma (27). Additionally, *KRAS-G12D* mutation can drive immunosuppression and enhance ICI resistance in NSCLC (28). We observed the methylation level of the *PCK2* promoter reduced as the pathological stage of lung adenocarcinoma patients progressed, and upon the expression pattern at the single-cell level, *PCK2* was seen to be negatively linked with EMT and hypoxia.

To summarise, we conducted a predictive analysis of related mechanism pathways, finding *PCK2* may play an important role in the response to oxidative stress-induced senescence, gene silencing, the cell cycle, reactive protein methylation of histone arginine, inhibition of telomere response, and apoptosis-induced DNA damage, all of which affect biological events in lung adenocarcinoma. Cancer researchers have identified cell cycle arrest as a major feature of cellular senescence that must be overcome. The prevention of EMT, tumor suppression, or tumor advancement are all outcomes of cellular senescence, which may be triggered by a wide range of stimuli such as telomere shortening, oncogenic activation, and treatment. Restoring epigenetically silenced *PCK2* inhibits renal cell carcinoma by promoting endoplasmic reticulum stress progression and increased sensitivity to sunitinib (29). Liu *et al.* (30) discovered that metabolic reprogramming of *PCK1* and *PCK2* promoted TCA catalysis, oxidative stress, and apoptosis, suggesting low expression levels of *PCK2* may contribute to better prognosis by triggering the senescent

Conclusions

Accumulating evidence suggests *PCK2* plays an important role in lung adenocarcinoma and has potential as a biomarker of disease progression through many mechanisms. Prediction findings and *PCK2* molecular features suggest *PCK2* improves the prognosis of lung adenocarcinoma patients by inducing senescence through

oxidative stress and inhibiting the immune escape of tumor cells. Further cytological investigations are required for appropriate verification and research, but the finding suggests a potential target for anticancer therapies for the effective treatment of lung adenocarcinoma.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-542/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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