PLK1 is A Promising Biomarker for the Prognosis of Lung Adenocarcinoma

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Abstract

Background: As one of the commonly occurred lung cancer, over 40% of lung cancer are lung adenocarcinoma (LUAD) in the world. However, due to the lack of effective treatment method, the prognosis of this disease is poor. In this study, we identified a novel treatment target for LUAD. Differentially expressed genes (DEGs) were extracted from 551 LUAD cases in the Cancer Genome Atlas (TCGA) database and used for Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis. Gene set variation analysis (GSVA) and the CIBERSORT method were applied for the estimation of the biological pathways and the tumor-infiltrating immune cells (TICs). Protein-protein interaction (PPI) network and Cox regression analyses were conducted for the analysis of DEGs. Quantitative real-time PCR (qPCR) and immunoblotting were used for the validation of DEGs.

Results: Genes related to cell cycle were sorted out. Positive association between cell cycle scores and clinical features including age, clinical stages and metastasis was found. Negative association between cell cycle scores and overall survival of LUAD patients was detected. In PPI and Cox analyses, PLK1 was estimated to be a factor for the prognostic predictions of LUAD patients. In LUAD patients, PLK1 was positively associated to the clinical stages and negatively associated to the overall survival. As suggested by CIBERSORT analysis, significant positive correlations were found between the transcription level of PLK1 and the function of CD8+ and activated memory CD4+ T cell. Meanwhile, significant negative correlation between the transcription level of PLK1 and activated natural killer cell was also found out. Moreover, PLK1 overexpression caused elevated immune cytotoxicity, including cytolytic activity score, IFN- γ score and IFN- γ level.

Conclusions: PLK1 displayed robust correlations with key features of TICs related to the immune-microenvironment of cancer, and therefore was suggested as a promising biomarker for the prognostic prediction of LUAD.

1 Introduction

Lung cancer is the most commonly occurred solid tumor worldwide.¹ Among all lung cancer cases, 85% cases are non-small cell lung carcinoma (NSCLC). NSCLC includes adenocarcinoma, squamous cell carcinoma and large cell carcinoma.^{2,3} Among them, the incidence of lung adenocarcinoma (LUAD) accounts for 2 third of all NSCLC cases.⁴ Despite recent developments in the multiple targeted treatments and new immunotherapies of LUAD, the 5-year prognosis of LUAD patients is still very poor.⁵ Such phenomenon is possibly caused by the early disseminations and metastasis of LUAD. Moreover, the mechanism underlying the onset and development of LUAD are unclear. Meanwhile, how the tumor-infiltrating immune cells (TICs) interact with LUAD is also poorly understood.

Previous researches report several LUAD progression-correlated genes. Elevated expression of the p53-inducible gene 3 (PIG3) is found to associated with incidence of lymph node metastasis in LUAD patients, and demonstrated to contribute to the development of LUAD through the enhancement of

FAK/Src/paxillin pathway activation.⁶ Enolase 1 (ENO1) is a protein coding gene for glycolysis enzyme related to glucose metabolism. It is also demonstrated to enhance LUAD tumor development. Latest research suggests that circ-ENO1 silencing causes the decrease in glycolysis, inhibits cell proliferation, and therefore promotes apoptosis through suppressing the transcription level of ENO1 in LUAD patients.⁷ Also, low expression level of ATM interactor (ATMIN) gene is reported to associated with LUAD. Heterozygous ATMIN deficiency is found to participate in promoted tumor proliferation and advance cancer grade in animal study of LUAD, suggesting the tumor suppressive effect of ATMIN in LUAD.⁸ Moreover, Polo-like Kinase 1 (PLK1) is previously demonstrated to associated with poor prognosis for LUAD patients.⁹ However, it is still not clear how transcription level of PLK1 associates with TICs.

Recently, accumulative evidences suggest the pivotal role played by TICs in regulating the malignance and impacting the treatment outcome of various types of cancer through the regulation of tumor microenvironment (TME).¹⁰⁻¹² Specifically, features immune cells are found to be reliable indicators for the treatment outcomes of patients.¹³⁻¹⁵ Tumor-infiltrating T cells are found in over half of patients with ovarian cancer. The present of tumor-infiltrating T cells is also found to significantly associated with better 5-year prognosis in ovarian cancer.¹⁶ Clinical follow-up study of triple-negative breast cancer (TNBC) indicates that better prognosis is associated with high CD8+ T cell number (over 14%).¹⁷ High tumor-infiltrating lymphocytes (TILs) level is also found to associate with low histologic grades of tumor.¹⁸ In LUAD, patients with high TILs are also found to have better prognostic predictions in both progression-free survival (PFS) and overall survival (OS).¹⁹

In this study, we aimed to demonstrate the differences of TICs between LUAD and healthy lung, confirm the correlation between hub genes and TICs, and unveiling the possible mechanism underlying the onset and development of LUAD. Workflow details of this study was listed as in the Figure 1.

2 Materials And Methods

2.1 DEGs extraction

Data of RNA-sequencing (HTSeq-FPKM) of LUAD patients were obtained from Cancer Genome Atlas (TCGA, <https://cancergenome.nih.gov/>). The dataset included 497 LUAD lung tissue samples and 54 normal samples from healthy controls. The GSE31210 gene transcription profile was obtained from Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/geo/>), containing 226 LUAD samples and 20 healthy controls. The R packages “edgeR” were used for the identification of DEGs. Normalization of GSE31210 matrix data were carried out by “limma”. Cutoff value for DEGs was set as adjusted p -value<0.05 with $|\log \text{ fold change}|>2$ in this study.

2.2 GSVA and KEGG enrichment analysis

The R packages “GSVA” were applied for Gene set variation analysis (GSVA) ($|\log \text{ fold change}|>0.3$ and adjusted p -value<0.05), and “clusterProfiler” was applied for Kyoto Encyclopedia of genes and Genomes

(KEGG) enrichment analysis (adjusted p -value<0.05). The “c2.cp.kegg.v7.1.symbols.gmt” dataset was obtained from Molecular Signature Database (<http://software.broadinstitute.org/gsea/msigdb/index.jsp>) and was used for normalization.

2.3 PPI network and Cox regression analysis

Protein-protein interaction (PPI) network analysis was carried out by Search Tool for the Retrieval of Interacting Genes database (STRING, <https://string-db.org/>) (cutoff value: combined score $\geq$ 0.4) and illustrated by Cytoscape (version 3.6.0, <https://cytoscape.org/>). The R package “survival” was applied for Cox regression analysis (p -value<0.001).

2.4 Survival and correlation analyses

The R packages “survival” and “survminer” were applied for estimating OS and PFS of patients with LUAD. The Kaplan-Meier method was used the generation of survival curves (significance was set as p -value<0.05). Subsequently, Kaplan-Meier Plotter (<https://kmplot.com/analysis/>) and Gene Expression Profiling Interactive Analysis (GEPIA) (<http://gepia.cancer-pku.cn/>) were conducted for survival analysis. The Pearson coefficient was applied for correlation analysis using SPSS 22.0.

2.5 Gene expression confirmation

Statistical analysis of Hub genes was carried out by TCGA dataset and GSE31210. Difference in hug gene expression pattern between LUAD and healthy controls was carried out by meta-analysis on Oncomine database website (<https://www.oncomine.org>). Illustration of the protein levels were carried out based on the immunohistochemical result of gene extracted from Human Protein Atlas (HPA, <https://www.proteinatlas.org>). Finally, both mRNA and protein level of targeted genes were confirmed using quantitative real-time polymerase chain reaction (qRT-PCR) and immunoblotting.

2.6 TME immune activities assessments

Estimation of the portions of TICs in LUAD was carried out by CIBERSORT. Heatmap of TICs correlation was generated by R package “corrplot”. Transcript Per Million (TPM) was adapted from the FPKM values of TCGA dataset. The immune cytolytic activity score (CAS) was evaluated according to the average values of GZMA and PRF1 level in TPM. Score of interferon-gamma (IFN- γ) was evaluated according to the average of CD8A, GZMA, GZMB, IFN- γ , EOMES, CXCL9, CXCL10 and TBX21 level in FPKM.

2.7 Cell cultures

BEAS, H1299, H1975, SPC-A-1, A549, and PC-9 cells were obtained from Shanghai Institute of Biology, the Chinese Academy of Sciences. All cell cultures were kept in high glucose DMEM media with 10% FBS and 1% penicillin/streptomycin (37°C, 5% CO₂).

2.8 qRT-PCR

Total RNA Extraction Kits (Solarbo) were used for the preparation of total RNA. cDNA was produced by first-strand cDNA synthesis kit (Invitrogen, USA). qRT-PCR was carried out on Applied Biosystems ABI

PRISM 7300 plus platform. Primers for PLK1 (Sense: 5'-AAAGAGATCCCGGAGGTCCTA-3'; Anti-sense: 5'-GGCTGCGGTGAATGGATATTTC-3') and GAPDH (Sense: 5'-GGAGCGAGATCCCTCCAAAAT-3'; Anti-sense: 5'-GGCTGTTGTCATACTTCTCATGG-3') were used.

2.9 Immunoblotting

Protein samples were prepared in SDS cell lysis, separated by 10% SDS-PAGE, and transferred to PVDF film (ThermoFisher, USA; 0.45 μ m). The films were blocked in 5% skimmed milk. Primary antibodies of PLK1 (1:1000 dilution, Abcam, ab189139) and β -actin (1:1000 dilution, Abcam, ab8226) were incubated with the membrane (4°C, ON). HRP-conjugated secondary antibody (Bioss) was then applied (RT, 1 hour). Positive staining was imaged by Tanon 5200.

2.10 Statistical analysis

All statistical analyses were carried out by R software (version 3.6.0) and SPSS (version 22.0). Pearson's correlation test was used for correlation analysis. Kaplan-Meier analysis was used for the comparison of statistical differences using log-rank test. Analysis of qRT-PCR and immunoblotting were carried out by Analysis of Variance (ANOVA). Each experiment was carried out in triplicates. Statistical significance was set as p -value < 0.05.

3 Results

3.1 Up-regulated signaling in LUAD

GSVA was conducted for the exploration of KEGG pathways according to data extracted from TCGA dataset. As compared to healthy controls, 48 KEGG signaling pathways (19 down-regulated and 29 up-regulated) were differentially expressed in LUAD (Figure 2A and **Supplementary Table S1**). The pattern of the results was illustrated as heatmap (Figure 2B). KEGG enrichment analysis of a total number of 1419 DEGs (310 down-regulated and 1,109 up-regulated) extracted from the TCGA dataset (Figure 2C) included cell cycle, protein digestion and absorption, ECM-receptor interaction, complement, coagulation cascades, and p53 signaling pathway (Figure 2D, **supplementary Table S2**). Change in signaling pathway related to cell cycle was commonly found in GSVA and KEGG enrichment studies (Figure 2E).

3.2 Cell cycle correlated with OS and clinical features of LUAD

TCGA dataset was studied for the evaluation of the potential usage of genes related to cell cycle as indicators for the prognostic prediction of LUAD. As suggested by the results, higher cell cycle score was found significantly related to LUAD patients (**Supplementary Figure S1**). Also, higher cell cycle scores were found in male patients ($p=0.0036$) and patients with N1-3 ($p=0.0007$) and M1 ($p=0.0186$). However, no significant difference of cell cycle score was found related to age or T classification subgroups (Figures 3A-E). Also, cell cycle scores were found to positively correlated with tumor stages of LUAD

patients (Figures 3F, G). We subsequently divided the LUAD patients by thier high or low cell cycle score, and found that the patients with high cell cycle score demonstrated significant association with poor OS and PFS (Figures 3H, I).

3.3 Combined analysis of PPI network and univariate Cox regression analysis

A total number of 23 DEGs related to signaling pathways of cell cycle were listed as in Table 1. PPI network analysis was carried out using those DEGs, and generated 23 nodes and 240 edges (Figure 4A). The connectivity degrees of genes were estimated and illustrated in the histogram (Figure 4B). In the results, DEGs including *BUB1B*, *CDC6*, *CDK1*, *MCM2*, *CDC20*, *CCNA2*, *CCNB1*, *CCNB2*, *CDC45*, *PLK1*, *CHEK1* and *CCNE1* were found to have the highest degrees of connectivity. Also, univariate Cox analysis identified that *CCNA2*, *PTTG1*, *CDC25C*, *CCNB1* and *PLK1* were found to correlated with the OS of LUAD patients (Figure 4C). *CCNA2*, *CCNB1* and *PLK1* were commonly found in the results of PPI network (12 genes) univariate Cox analysis (5 genes) (Figure 4D).

Table 1
Differentially expressed genes in the cell cycle pathway

Gene	logFC	p-value	adjust p-value	Gene	logFC	p-value	adjust p-value
<i>CDC20</i>	3.681	8.76E-47	3.21E-45	<i>PKMYT1</i>	2.599	9.12E-26	1.29E-24
<i>CDKN2A</i>	3.299	9.88E-19	8.65E-18	<i>ESPL1</i>	2.566	7.64E-21	7.91E-20
<i>CDC6</i>	3.140	4.56E-35	1.05E-33	<i>ORC6</i>	2.528	1.12E-23	1.40E-22
<i>BUB1B</i>	3.042	2.42E-32	4.84E-31	<i>BUB1</i>	2.508	5.02E-28	8.14E-27
<i>CCNE1</i>	3.000	1.78E-24	2.34E-23	<i>ORC1</i>	2.431	6.50E-23	7.74E-22
<i>PLK1</i>	2.998	5.85E-37	1.45E-35	<i>CDK1</i>	2.424	1.63E-30	3.01E-29
<i>CCNB1</i>	2.946	2.33E-44	7.85E-43	<i>MAD2L1</i>	2.384	2.76E-24	3.61E-23
<i>CCNB2</i>	2.921	2.60E-37	6.54E-36	<i>PTTG1</i>	2.305	3.80E-32	7.53E-31
<i>CDC45</i>	2.894	2.51E-30	4.56E-29	<i>MCM4</i>	2.219	1.60E-37	4.05E-36
<i>TTK</i>	2.849	1.96E-25	2.71E-24	<i>CHEK1</i>	2.069	2.99E-22	3.41E-21
<i>CDC25C</i>	2.811	9.20E-23	1.09E-21	<i>MCM2</i>	2.014	2.82E-29	4.81E-28
<i>CCNA2</i>	2.791	4.82E-34	1.04E-32				

Abbreviation: FC, fold change.

3.4 High PLK1 in LUAD

PLK1 was found significantly highly expressed in LUAD patients compared to that in healthy controls (Figures 5A, B). Meta-analysis suggested elevated PLK1 level associated with LUAD in 5 datasets (Beer Lung,²⁰ Garber Lung,²¹ Hou Lung,²² Stearman Lung,²³ and Su Lung,²⁴ Figure 5C). Also, revealed by the immunohistochemical images from HPA, PLK1 level was found elevated in tissue samples collected from LUAD patients compared to those in healthy controls (Figure 5D). In the analysis of GSE31210 profile, PLK1 expressive level was also suggested to be significantly higher in LUAD samples than in healthy controls (Figure 5E). Moreover, as validated by qRT-PCR and immunoblotting analysis, the transcription and protein level of PLK1 were found increased in H1975 and SPC-A-1 cell than BEAS cells (Figures 5F, G).

3.5 PLK1 for prognosis of LUAD

Survival analysis and Cox regression analysis were carried out for the evaluation *PLK1* as prognostic marker. We found that high *PLK1* expression significantly indicated poor OS of LUAD patients (Figures 6A-C). The LUAD patients with high *PLK1* expression were negatively associated with PFS (Figures 6D-F). Meanwhile, the results of Cox analysis suggested that *PLK1* could be used as independent prognostic prediction marker for patients with LUAD (Table 2). The *PLK1* expressive level was significantly increased in male versus female, T2-4 versus T1-2, N1-3 versus N0 and M1 versus M0 subgroups (Figures 7A-D), but not in age subgroups (**Supplementary Figure S2**). Besides, PLK1 expressive level demonstrated positive correlation with tumor stages in LUAD (Figures 7E, F). The LUAD patients were divided to 2 subgroups of high or low median PLK1 expressive level. High PLK1 expression was significantly related to poor OS (Figures 7G-P) and worse PFS (**Supplementary Figure S3**) in female, male, T1-2, N0, M0, stage I-II, and stage III-IV subgroups, but not in T3-4, N1-3 and M1 subgroups.

Table 2
Cox regression analysis for PLK1 expression on OS and PFS of LUAD patients.

Parameter	Univariate Cox analysis			Multivariate Cox analysis		
	HR	95%CI	pvalue	HR	95%CI	pvalue
TCGA (OS)						
gender	1.035	0.717~1.495	0.852	0.927	0.638~1.347	0.691
stage	1.654	1.401~1.951	<0.001	2.037	1.275~3.254	0.003
T classification	1.632	1.315~2.024	<0.001	1.189	0.940~1.503	0.149
M classification	1.757	0.964~3.203	0.066	0.353	0.103~1.210	0.098
N classification	1.790	1.459~2.196	<0.001	0.970	0.651~1.444	0.881
age	1.002	0.983~1.021	0.843	1.013	0.993~1.033	0.192
<i>PLK1</i>	1.046	1.020~1.073	0.001	1.053	1.023~1.084	0.001
TCGA (PFS)						
age	1.005	0.986~1.024	0.615	1.016	0.996~1.036	0.120
gender	1.131	0.784~1.630	0.511	0.981	0.675~1.425	0.919
stage	1.672	1.411~1.981	<0.001	1.575	1.031~2.405	0.036
T classification	1.573	1.285~1.926	<0.001	1.208	0.957~1.525	0.112
M classification	2.146	1.173~3.927	0.013	0.707	0.241~2.074	0.527
N classification	1.737	1.415~2.132	<0.001	1.130	0.789~1.619	0.505
<i>PLK1</i>	1.052	1.026~1.079	<0.001	1.057	1.028~1.086	<0.001
GSE31210 (OS)						
age	1.025	0.977-1.075	0.306	1.035388	0.985-1.088	0.169
gender	1.519	0.780-2.955	0.219	1.100991	0.547-2.215	0.787
stage	4.232	2.175-8.236	<0.001	3.720136	1.886-7.337	<0.001
<i>PLK1</i>	1.658	1.192-2.306	0.003	1.538064	1.068-2.215	0.021
Abbreviation: LUAD, lung adenocarcinoma; HR, hazard ratio; CI, confidence interval; OS, overall survival; PFS, progression-free survival.						

3.6 PLK1 Associates with immune responses of TME

For the investigation of the association of PLK1 level in the immune-microenvironment of LUAD, TCGA dataset were analyzed focusing on TICs, CAS and IFN- γ score. Using CIBERSORT algorithm, the fraction

of 21 subtypes of infiltrating immune cells was estimated as illustrated in the **Supplementary Figure S4**. TIC profiles were visualized by histogram, meanwhile the correlations between TICs were demonstrated in the heatmap. Significant difference was found in the proportion scores of the 12 subtypes of TICs between high and low PLK1 level groups (Figure 8A). Also, as revealed by the Pearson correlation analysis, 13 subtypes of TICs were significantly related to PLK1 level (Figure 8B). The 12 subtypes of TICs were commonly identified (Figure 8C and **Supplementary Table S3**). The generated results of changed TICs were identified as the PLK1-associated TICs. In the results, the expressive levels of CAS and IFN- γ , scores of IFN- γ were increased in LUAD patients (Figures 9A-C). Moreover, the expressive levels of CAS and IFN- γ , scores of IFN- γ were found higher in LUAD patients with high PLK1 level (Figure 9D-F). Significant associations were found between the expressive levels of PLK1 and CAS, IFN- γ and scores of IFN- γ (Figures 9G-I).

4 Discussions

LUAD was one of the cancers with the highest mortalities.²⁵ In clinic, surgeries were the most widely used method for LUAD treatment, which was mainly facilitated by early diagnosis. Combined use of surgery and chemotherapy treatments increased 5-10% survival rates.²⁶ However, the survival rates for LUAD patients were unsatisfactory. Poorly understood mechanisms related to the onset and development of LUAD also significantly limited the discoveries of novel effective treatment method for LUAD patients. In this study, we found that PLK1 was a hug gene related to cell proliferation which was positively correlated with the clinical stages and cytotoxic activities in the TME.

PLK1 was involved in the regulation of cell cycle through several pathways, including functioning as a G2/M checkpoint, participating the regulation of centrosome, spindle assemblies and chromosome segregations.²⁷ Previous researches found that high level of PLK1 was associated with the incidence of malignant tumor. Meanwhile, the overexpression of PLK1 negatively impacted the prognosis of patients with different types of cancers.²⁸⁻³⁰ Large amount of evidences suggested the association of high PLK1 transcription and protein level with poor prognosis and treatment outcomes in gastric tumor.³¹ High PLK1 level was also found strongly correlated with ovarian clear cell carcinoma (OCCC). PLK1 inhibiting promoted the sensitivities of OCCC cell to the cisplatin treatments through the enhancement of autophagy and apoptosis *in vitro*.³² High expressive level of PLK1 was found to associate with TNBC according to a clinical study containing 3,173 samples. Overexpression of PLK1 significantly correlated to TICs and poor prognostic prediction of the patients.²⁹ Gene set enrichment study also found that genes related to cell cycle and MYC targets were significantly enriched in TNBC patients with high PLK1 expressive level. In *in vitro* study, PLK1 was demonstrated to promote the signaling of transforming growth factor (TGF)- β and therefore increased the invasiveness of cancer cells. This result was consistent with the clinical findings that the expression PLK1 robustly predicted the survival of patients with metastatic NSCLC.³³ Moreover, PLK1 was also suggested as a potentially effective treatment target of various types of cancer due to the suppressive effects on cell growth and promoting effects on apoptosis of tumor cells via the inhibition of PLK1 expression.³⁴⁻³⁶

TICs was found to contribute to the regulation of the immune-microenvironment of tumor, and therefore impacted on the development of tumor and the treatment outcomes of patient with cancers.^{37–39} High tumor-infiltrating CD4+ and CD8+ T cell levels in pancreatic cancer strongly indicated longer survival time.⁴⁰ Two subsets of tumor-infiltrating CD4+ and CD8+ T cells were found in NSCLC respectively expressing CD69 and CD103, both of which were markers of resident memory T cells.⁴¹ Other than functioning as memory cells ready for quick responses to secondary infections, CD103+ T cells was also demonstrated to target tumor cells in previous studies.^{42,43} Programmed cell death-1 (PD-1), CD244 and cytotoxic T-lymphocyte Antigen-4 (CTLA-4) were majorly expressed on both CD8+ and CD4+ T cells. Meanwhile, both tumor necrosis factor alpha (TNF- α) and IFN- γ were predominantly produced by CD103+ CD4+ T cells.^{44–47} Also, cytotoxic T cells were able to produce cytolytic effectors including perforin (PRF1) and pro-apoptotic granzymes (GZMA), and participated the immune reactions against tumor cells through the action of granzyme-perforin pathway.⁴⁸ Previous study also demonstrated that the dominant cytotoxic effects of CD56dim natural killer (NK) cells against tumor cells in advanced head and neck squamous cell carcinoma (HNSCC). In HNSCC patients, CD56dim NK cells were found to robustly correlated to promising prognostic predictions.⁴⁹ Moreover, both infiltrating NK cells and macrophages were found to indicate good survival rates in patients with stage II and III esophageal cancer.⁵⁰ Most importantly, TME was demonstrated to produce strong impact on the cytotoxic effects of NK cells against tumor cells possibly through its interaction with various signaling pathways related to the activation and infiltration of NK cells.^{51,52} Moreover, the immune-regulatory effects mediated by TME was found in the suppression of infiltrating DCs in maintaining the homeostasis of CD8+ T cell immuno-reaction and tumor antigen tolerance, leading to the promotion of tumor growth.⁵³ For this reason, the immune-regulatory effect of TME and the molecular mechanisms underlying this effect were proposed as a pivotal factor in the development of new immunotherapy approaches of various cancers.

In this study, we found that the expressive level of PLK1 was correlated to the clinical stages and the regulations of immune microenvironment of tumor cells. However, our findings were limited due to the following reasons. LUAD cell lines were used for the qRT-PCR and immunoblotting verifications of PLK1 level. Therefore, further evidences in *in vivo* and clinical studies were necessary for the validations of the mechanisms suggested by this study. Also, lack of verifications in clinical samples in this study limited the reliability of our findings. Moreover, the correlation studies of clinical features were unsatisfactory caused by the unbalanced clinical data obtained from the available datasets. Finally, in our results, the underlying mechanisms of the association between PLK1 and TICs were not fully understood.

5 Conclusions

Our findings provided evidences for a further understanding of the onset and development of LUAD. As suggested by our results, PLK1 level could potentially be used as both new treatment target and novel prognosis biomarkers in LUAD patients. PLK1 was found to robustly correlated with the function of TICs in TME of tumor cells. Further studies were needed for validations and mechanism investigations of the role played by PLK1 in the regulation of TICs in LUAD.

Abbreviations

LUAD: lung adenocarcinoma; DEGs: Differentially expressed genes; TCGA: The cancer genome atlas; GSVA: Gene set variation analysis; TICs: Tumor-infiltrating immune cells; PPI: Protein-protein interaction; TME: Tumor microenvironment; TILs: Tumor-infiltrating lymphocytes; NSCLC: Non-small cell lung carcinoma; PLK1: Polo-like Kinase 1; PFS: Progression-free survival; OS: Overall survival.

Declarations

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from The Cancer Genome Atlas database (<https://cancergenome.nih.gov/>).

Ethics approval and consent to participate

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Authors' contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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Figures

Figure 2

GSVA for samples and KEGG enrichment analysis for DEGs. (A) 29 upregulated (red spots) and 19 downregulated (green spots) metabolic pathways were identified via GSVA in LUAD samples versus normal lung samples. Adjusted p -value < 0.05 and $|\log FC| > 0.3$ were utilized as the cutoff criteria for determining significant differences. (B) Heatmap for the significantly altered metabolic pathways. (C) 1,109 upregulated (red spots) and 310 downregulated (green spots) DEGs were identified in LUAD

samples versus normal lung samples. Adjusted p-value<0.05 and |logFC|>2 were utilized as the cutoff criteria for determining significant differences. (D) KEGG enrichment analysis for DEGs. (E) Cell cycle was the common pathway identified in both GSEA and KEGG analyses.

Figure 3

Correlation of Cell Cycle score with clinical characteristics and survival of LUAD patients. Statistical difference for Cell Cycle score in LUAD patients with clinical traits, including age (A), gender (B), primary

tumor (C), lymph node metastasis (D), distant metastasis classification (E), and tumor stage (F). The correlation of Cell Cycle score with tumor stage (G). Kaplan-Meier curves for overall survival (OS) (H) and progression-free survival (PFS) (I) in high and low score patients grouped by the median of Cell Cycle score.

Figure 4

PPI network and univariate Cox analysis for the 23 DEGs in the cell cycle pathway. (A) The PPI network consisted of 23 nodes (genes) and 240 edges (interactions between genes). (B) Histogram of the

connectivity degrees of genes in the PPI network. (C) Univariate Cox analysis for the 23 DEGs. A p-value<0.001 was utilized as the cutoff criterion. (D) Venn diagram indicating 3 common genes which overlapped the 12 leading genes in the PPI network and the 5 prognosis-related genes in the Cox analysis.

Figure 5

Validation of PLK1 expression. PLK1 expression is significantly upregulated in LUAD samples compared to normal lung samples in both unpaired (A) and paired (B) difference analyses based on the TCGA

dataset. PLK1 expression is significantly increased in LUAD samples compared with normal samples in a meta-analysis (C), immunohistochemical analysis (D) and GSE31210 profile (E). Gene expression level (F) and protein expression level (G) of PLK1 were significantly increased in H1975 and SPC-A-1 cell lines compared with BEAS cells. n = 3. Data represent mean $\pm$ SEM, *p < 0.05, **p < 0.01, ***p < 0.001 vs. BEAS.

Figure 6

Correlation of PLK1 expression with the survival of LUAD patients. High PLK1 expression negatively associated with OS in LUAD according to TCGA dataset (A), GEPIA database (B) and Kaplan-Meier plotter platform (C). High PLK1 expression predicted worse PFS relative to TCGA dataset (D), GEPIA database (E) and Kaplan-Meier plotter platform (F).

Figure 7

Prognostic significance of PLK1 in LUAD. Statistical difference for PLK1 expression in LUAD patients with clinical traits, including gender (A), primary tumor (B), lymph node metastasis (C), distant metastasis classification (D), and tumor stage (E). The correlation of PLK1 expression with tumor stage (F). Kaplan-Meier curves for the OS in LUAD subgroups with female (G), male (H), T1-2 (I), T3-4 (J), N0 (K), N1-3 (L),

M0 (M), M1 (N), stage I-II (O), and stage III-IV (P). LUAD patients were divided into high and low HMMR expression subgroups relevant to the median of HMMR expression levels.

Figure 8

Correlation of PLK1 expression with TICs proportion in LUAD patients. (A) Violin plot of the relative proportion of TICs in the high and low PLK1 expression subgroups grouped by median PLK1 expression. Green-and red-colored columns represent the low and high PLK1 expression subgroups, respectively. (B)

Scatter plots for the significant correlation of the 13 TICs with PLK1 expression. (C) Venn diagram indicating 12 kinds of common TICs overlapped by difference analyses and correlation tests.

Figure 9

Correlation of PLK1 expression with cytotoxic function. Histogram for cytolytic activity score (A), IFN-γ expression level (B), and IFN-γ score (C), which markedly increased in LUAD samples versus normal lung samples. Histogram for cytolytic activity score (D), IFN-γ expression level (E), and IFN-γ score (F) were significantly higher in the high PLK1 expression subgroup than the low PLK1expression subgroup. Values displayed were divided by median PLK1 expression. Scatter plots for cytolytic activity score (G), IFN-γ expression level (H), and IFN-γ score (I), which were significantly correlated with PLK1 expression.

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