The identification and prediction of lung adenocarcinoma prognosis using a novel gene signature associated with DNA replication

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Background: Lung adenocarcinoma (LUAD) is the most aggressive lung cancer phenotype, and patients' clinical response is often limited by primary or acquired mechanisms of resistance to oncological therapy. One of the current clinical needs is to define clinical predictors for the prognosis of LUAD, aiming at offering patients a persistent treatment likely to delay disease progression as much as possible. This study relies on data from The Cancer Genome Atlas (TCGA) to define the functional roles and prognostic implications of DNA replication-related genes in LUAD.

Methods: Clinical features and RNA-sequencing data were collected from 607 LUAD patients from TCGA-LUAD dataset, with the aims to identify the genes related to patient prognosis, and the pathways related to DNA replication in LUAD.

Results: A total of 2,412 prognostic genes were obtained, and the DNA replication-related pathways closely associated with LUAD were identified by a Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. TCGA-LUAD patients were divided into a high- (G1) and low- (G2) risk groups based on the 15 DNA replication-related genes (i.e., *FEN1*, *MCM5*, *POLD2*, *MCM4*, *MCM6*, *SSBP1*, *POLE2*, *RFC2*, *MCM2*, *PCNA*, *POLA2*, *MCM7*, *RFC3*, *POLE4*, and *RPA3*). The upregulated genes were mainly related to the hallmarks of cancer (e.g., chromosome segregation, DNA replication, the cell cycle checkpoint, and DNA helicase activity), while the downregulated genes were mainly related to leukocyte activation involved in inflammatory macrophage activation, and passive transmembrane transporter activity. The immune cells, including the B cells, endothelial cells, natural killer (NK) cells, cluster of differentiation (CD)4^{+} T cells, and CD8^{+} T cells, of the Group 1 (G1) LUAD samples were clearly different from those of the Group (G2) LUAD samples. In addition, 5 of the 10 immune checkpoint inhibitor (ICI)-related genes (i.e., *CD274*, *LAG3*, *PDCD1*, *PDCD1LG2*, and *SIGLEC15*) were of a higher level in the G1 LUAD samples than in the G2 LUAD samples. The tumor stemness of the two risk groups differed significantly. Furthermore, a six-gene (*FEN1*, *MCM5*, *POLD2*, *MCM4*, *SSBP1*, and *POLE4*) prognostic model was constructed to predict the prognosis of LUAD patients.

Conclusions: There is a close relationship between the DNA replication-related genes and the tumor classification of LUAD patients. An innovative signature related to DNA replication was found to be a good prognostic predictor of LUAD. Our findings may provide novel insights into the diagnosis and treatment of LUAD.

Keywords: Lung adenocarcinoma (LUAD); prognosis; DNA replication; gene signature; biomarkers

Introduction

Lung cancer is the most prevalent and lethal type of cancer, and lung adenocarcinoma (LUAD) accounts for approximately 60% of non-small cell lung cancer cases (1,2). Patients with LUAD usually undergo treatments such as surgery, chemotherapy, radiation therapy, and molecular targeted therapy. However, due to rapid tumor growth and resistance to existing treatments, only about 30% of LUAD patients survive beyond five years (1,3). Consequently, it is critical to enhance understanding of the mechanisms underlying LUAD.

In addition to curative surgeries like lobectomy or segmental lung resection with or without adjuvant chemotherapy, or radiotherapy, more precise treatments like epidermal growth factor receptor (EGFR)-targeted therapy and immunotherapy have enhanced the survival of LUAD patients (4). Moreover, Kirsten rat sarcoma 2 viral oncogene homolog (KRAS) mutant lung cancer is the most prevalent form of lung adenocarcinoma (LUAD) (3). However, the prognosis of LUAD patients continues to be unfavorable, and the 5-year overall survival (OS) rate of LUAD patients is still only approximately 16% (1-4). Based on the most recent the tumor-node-metastasis (TNM) staging system, the 5-year recurrence rates vary from around 20% in stage I to 50% in stage III (5,6). Thus, it is essential to extend our understanding of the potential mechanisms driving LUAD progression to improve patient prognosis.

Genome instability is a characteristic feature of cancer, and DNA replication is the cellular process most susceptible to it (7). High levels of DNA damage can lead to replication stress, causing genome instability (7). A novel and promising therapeutic strategy focusing on enhancing the integration of damaged deoxynucleoside triphosphates (dNTPs) in cancer cells has been suggested. Targeting NUDT1 (also known as MTH1), a protein that prevents the misincorporation of oxidized dNTPs during replication and is essential in cancer cells but dispensable in normal cells, can lead to the selective killing of cancer cells (7-9). Understanding the molecular mechanisms underlying replication stress is essential in understanding tumorigenesis. We performed a systematic analysis to assess the levels of DNA replication expression in LUAD, examined the relationship between DNA replication and the tumor immune microenvironment, and evaluated its prognostic significance. We present this article in accordance with the TRIPOD reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-2024-2536/rc)

Methods

The Cancer Genome Atlas (TCGA)-LUAD dataset

TCGA-LUAD dataset was obtained from TCGA database (https://portal.gdc.cancer.gov/), and the LUAD RNA-sequencing data were analyzed. The study was conducted in

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Highlight box

Key findings
- DNA replication is the cellular process most susceptible to DNA damaging and to risk of carcinogenesis. DNA replication-related genes classification may serve as good prognostic indicators of lung adenocarcinoma (LUAD).

What is known, and what is new?
- LUAD can be evaluated using DNA replication-related genes.
- This study identified LUAD subtypes and biomarkers associated with DNA replication.

What is the implication, and what should change now?
- Our results offer new perspectives for the diagnosis and therapeutic strategies of LUAD, potentially advancing clinical approaches to this disease.

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accordance with the Declaration of Helsinki (as revised in 2013).

Identification of prognostic genes in LUAD

A univariate Cox analysis was conducted to identify the genes associated with the prognosis of LUAD patients. Based on P values, the top 20 genes were shown. Using the R package ClusterProfiler, a Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was conducted to identify the signaling pathways associated with the prognostic genes in LUAD.

Identification of tumor subtypes based on the DNA replication-related pathways in LUAD

Consensus ClusterPlus (version 1.54.0) was used to perform a consensus clustering analysis of TCGA-LUAD dataset to investigate the expression of the DNA replication-related genes and LUAD subtypes. Using the R package pheatmap (version 1.0.12), a heatmap was generated by setting the cluster variable (k) from 2 to 6. A Kaplan-Meier analysis was conducted to compare the OS between the subgroups.

Identification of DNA replication-related differentially expressed genes (DEGs) in LUAD

The DEseq2 R package was used to identify the DEGs between the different LUAD clusters. A P value <0.05 and $\log_2$ fold change >1 was considered significant. For the visualization of the DEGs, the ggplot2 R package was used to create volcano plots. Based on data from the pheatmap R package (version 1.0.12), a heatmap of the DEGs was generated. To analyze the Gene Ontology (GO) and KEGG terms, the ClusterProfiler package was used.

Immune activity between the two DNA replication-related clusters in LUAD

An assessment of immune activity was conducted by CIBERSORT using the genes related to DNA replication. Using eight immune checkpoint-related genes (i.e., *CD274*, *PDCD1*, *PDCD1LG2*, *CTLA4*, *LAG3*, *HAVCR2*, *TIGIT*, and *SIGLEC15*), two DNA replication clusters were compared in terms of immune activity. Heatmaps and boxplots were generated using the R packages heatmaps and ggplot2. The infiltration of immune cells and the activation of immune pathways were compared using the Wilcox test. A P value <0.05 was considered statistically significant.

Development of the DNA replication-related gene prognostic model

TCGA-LUAD cohort prognostic model construction was carried out using the "glmnet" R package to evaluate the prognostic value of the DNA replication-related genes. The lambda condition was determined by the minimum criteria for variables with non-zero coefficients. The risk score was calculated using the following formula: Risk score = the expression level of each gene × the corresponding coefficient. Patients in TCGA-LUAD dataset were classified into high- and low-subgroups based on the median risk score. Using the "survival" R package, we compared the OS among high- and low-risk groups. A Cox proportional hazards analysis was conducted to determine the hazard ratios (HRs) with confidence intervals (CIs).

Statistical analysis

A survival analysis was performed to determine independent prognostic factors for LUAD, establishing a significance threshold at P<0.05. After adjusting for multiple comparisons, two-tailed P values below 0.05 were considered statistically significant.

Results

Identification of KEGG pathways and prognostic genes in LUAD

A univariate Cox analysis was conducted to identify the genes related to prognosis in LUAD patients. A total of 2,412 prognostic genes for LUAD were examined in the univariate Cox analysis. The top 20 genes are depicted according to their P values (*Figure 1A*). A KEGG enrichment analysis was then conducted using these prognostic genes for LUAD. The KEGG enrichment analysis indicated that pathways such as metabolic pathways, DNA replication, proteasome, and the cell cycle may play significant roles in LUAD (*Figure 1B*). Given the close association between DNA replication and cancer, our investigation then evaluated the functional implications of DNA replication status in patients with LUAD.

LUAD subtypes based on the DNA replication-related genes

DNA replication has been found to be closely associated with tumor development (7-9), but further research is
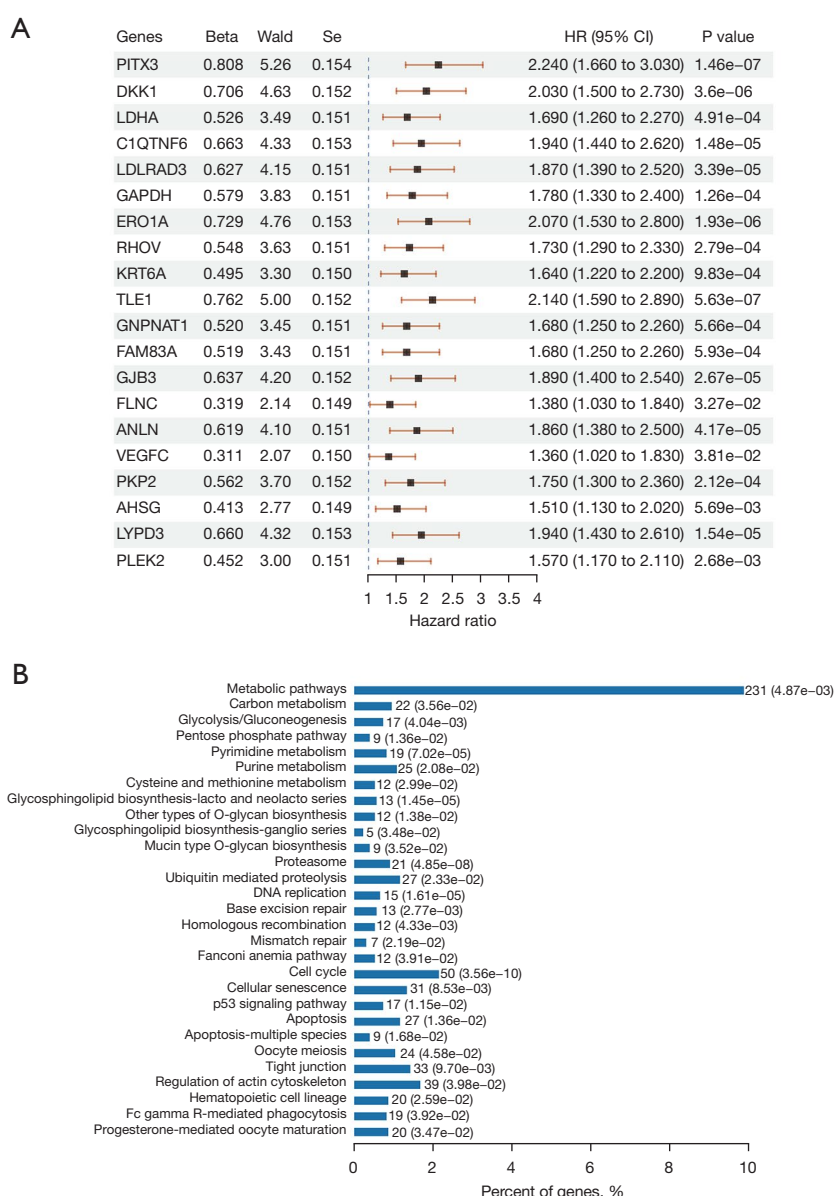
A

Genes	Beta	Wald	Se		HR (95% CI)	P value
PITX3	0.808	5.26	0.154		2.240 (1.660 to 3.030)	1.46e−07
DKK1	0.706	4.63	0.152		2.030 (1.500 to 2.730)	3.6e−06
LDHA	0.526	3.49	0.151		1.690 (1.260 to 2.270)	4.91e−04
C1QTNF6	0.663	4.33	0.153		1.940 (1.440 to 2.620)	1.48e−05
LDLRAD3	0.627	4.15	0.151		1.870 (1.390 to 2.520)	3.39e−05
GAPDH	0.579	3.83	0.151		1.780 (1.330 to 2.400)	1.26e−04
ERO1A	0.729	4.76	0.153		2.070 (1.530 to 2.800)	1.93e−06
RHOV	0.548	3.63	0.151		1.730 (1.290 to 2.330)	2.79e−04
KRT6A	0.495	3.30	0.150		1.640 (1.220 to 2.200)	9.83e−04
TLE1	0.762	5.00	0.152		2.140 (1.590 to 2.890)	5.63e−07
GNPNAT1	0.520	3.45	0.151		1.680 (1.250 to 2.260)	5.66e−04
FAM83A	0.519	3.43	0.151		1.680 (1.250 to 2.260)	5.93e−04
GJB3	0.637	4.20	0.152		1.890 (1.400 to 2.540)	2.67e−05
FLNC	0.319	2.14	0.149		1.380 (1.030 to 1.840)	3.27e−02
ANLN	0.619	4.10	0.151		1.860 (1.380 to 2.500)	4.17e−05
VEGFC	0.311	2.07	0.150		1.360 (1.020 to 1.830)	3.81e−02
PKP2	0.562	3.70	0.152		1.750 (1.300 to 2.360)	2.12e−04
AHSG	0.413	2.77	0.149		1.510 (1.130 to 2.020)	5.69e−03
LYPD3	0.660	4.32	0.153		1.940 (1.430 to 2.610)	1.54e−05
PLEK2	0.452	3.00	0.151		1.570 (1.170 to 2.110)	2.68e−03

Hazard ratio

B

Figure 1 Identification of KEGG pathways and prognostic genes in LUAD. (A) The prognostic genes in LUAD. (B) The KEGG enrichment pathways of the prognostic genes in LUAD. CI, confidence interval; HR, hazard ratio; KEGG, Kyoto Encyclopedia of Genes and Genomes; LUAD, lung adenocarcinoma; SE, standard error.

needed to determine the role of DNA replication in LUAD. A consensus clustering analysis was carried out using the identified DNA replication-related genes (i.e., *FEN1, MCM5, POLD2, MCM4, MCM6, SSBP1, POLE2, RFC2, MCM2, PCNA, POLA2, MCM7, RFC3, POLE4,* and *RPA3*) to further stratify the TCGA-LUAD patient cohort.

A consistency cluster analysis and a principal component analysis of the TCGA-LUAD patient cohort were conducted, and two clusters were distinguished (*Figure 2A,2B*). The gene expression profile of 15 DNA replication-related genes was shown in a heatmap between the two risk groups of TCGA-LUAD patients, the high- (G1) and low- (G2) risk group (*Figure 2C*). A statistically significant difference was found between clusters 1 and 2 in terms of OS (HR: 1.532, 95%
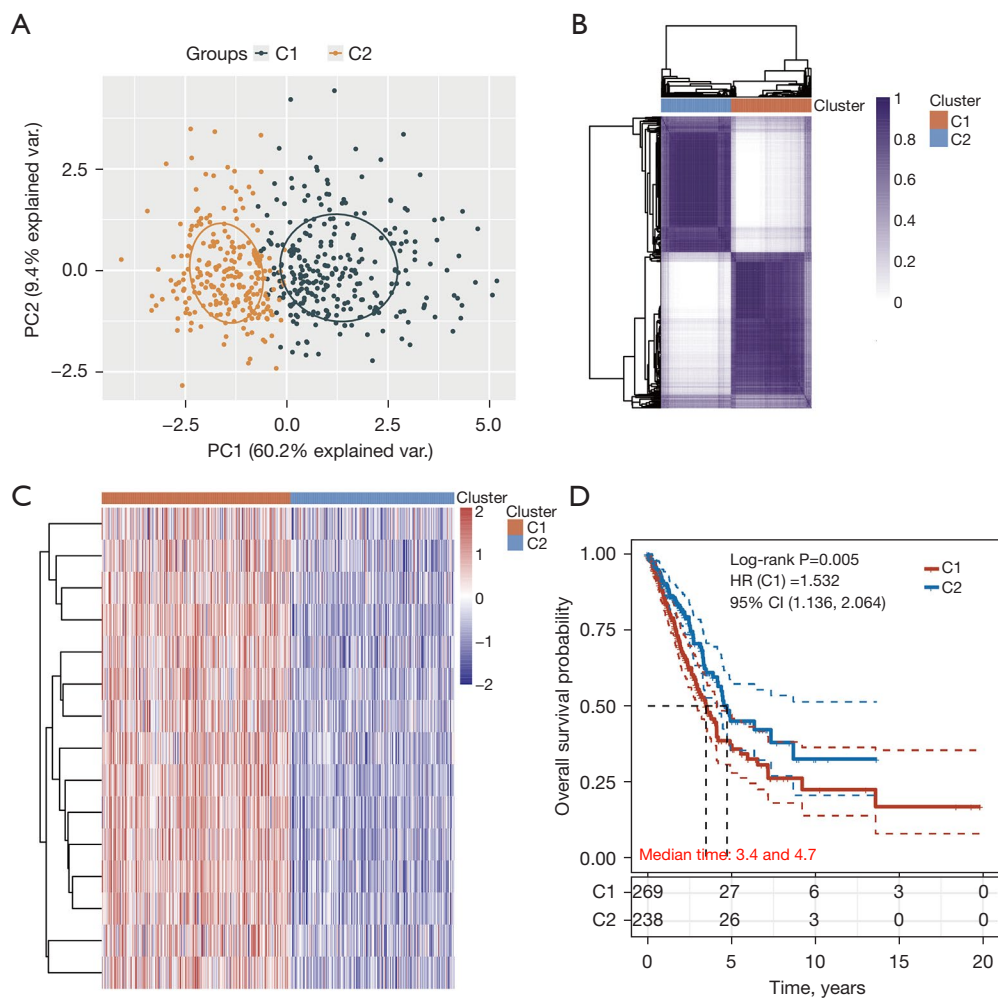
Figure 2 LUAD subtypes based on the DNA replication-related genes. (A) Two clusters of TCGA-LUAD patients were analyzed using a principal component analysis. (B) The consensus clustering matrix of two clusters in LUAD. (C) Heatmap of two clusters in LUAD. (D) Kaplan-Meier OS curves for the two clusters. CI, confidence interval; HR, hazard ratio; LUAD, lung adenocarcinoma; OS, overall survival; PC, principal component; TCGA, The Cancer Genome Atlas.
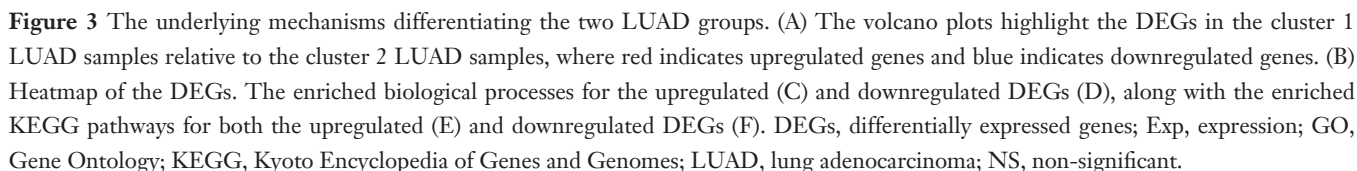
CI: 1.136–2.064, P=0.005; *Figure 2D*).

Identification of the underlying mechanisms between the two risk groups of LUAD patients

To examine the distinct mechanisms underlying the two risk groups, we identified the DEGs using the following criteria: P<0.05 and |log₂ fold change| >1. The results were depicted in a volcano plot [Group 1 (G1) *vs.* Group 2 (G2); *Figure 3A*]. The expression patterns of the top 50 DEGs demonstrated diverse trends across the two clusters as shown in the heatmap (*Figure 3B*).

To further explore the biological significance of these

DEGs, we performed GO and KEGG enrichment analyses. The results of the GO analysis for the biological processes revealed that the upregulated genes were mainly related to the hallmarks of cancer initiation and progression (e.g., chromosome segregation, DNA replication, the cell cycle checkpoint, and DNA helicase activity) (*Figure 3C*), while the downregulated genes were mainly related to leukocyte activation involved in inflammatory macrophage activation, and passive transmembrane transporter activity (*Figure 3D*). Similarly, the results of the KEGG analysis showed that the upregulated genes were primarily associated with the cell cycle, p53 signaling pathway, DNA replication, and base excision repair (*Figure 3E*), while the downregulated

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Figure 3 The underlying mechanisms differentiating the two LUAD groups. (A) The volcano plots highlight the DEGs in the cluster 1 LUAD samples relative to the cluster 2 LUAD samples, where red indicates upregulated genes and blue indicates downregulated genes. (B) Heatmap of the DEGs. The enriched biological processes for the upregulated (C) and downregulated DEGs (D), along with the enriched KEGG pathways for both the upregulated (E) and downregulated DEGs (F). DEGs, differentially expressed genes; Exp, expression; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; LUAD, lung adenocarcinoma; NS, non-significant.

genes were primarily associated with arachidonic acid metabolism, linoleic acid metabolism, and complement and coagulation cascades (*Figure 3F*). Many studies have shown that chromosome segregation, DNA replication, and the

cell cycle checkpoint are tumor markers (10,11). The above results indicated that the tumor migration and proliferation ability of the tumor cells tend to be stronger in the G1 LUAD subtype than the G2 LUAD subtype.
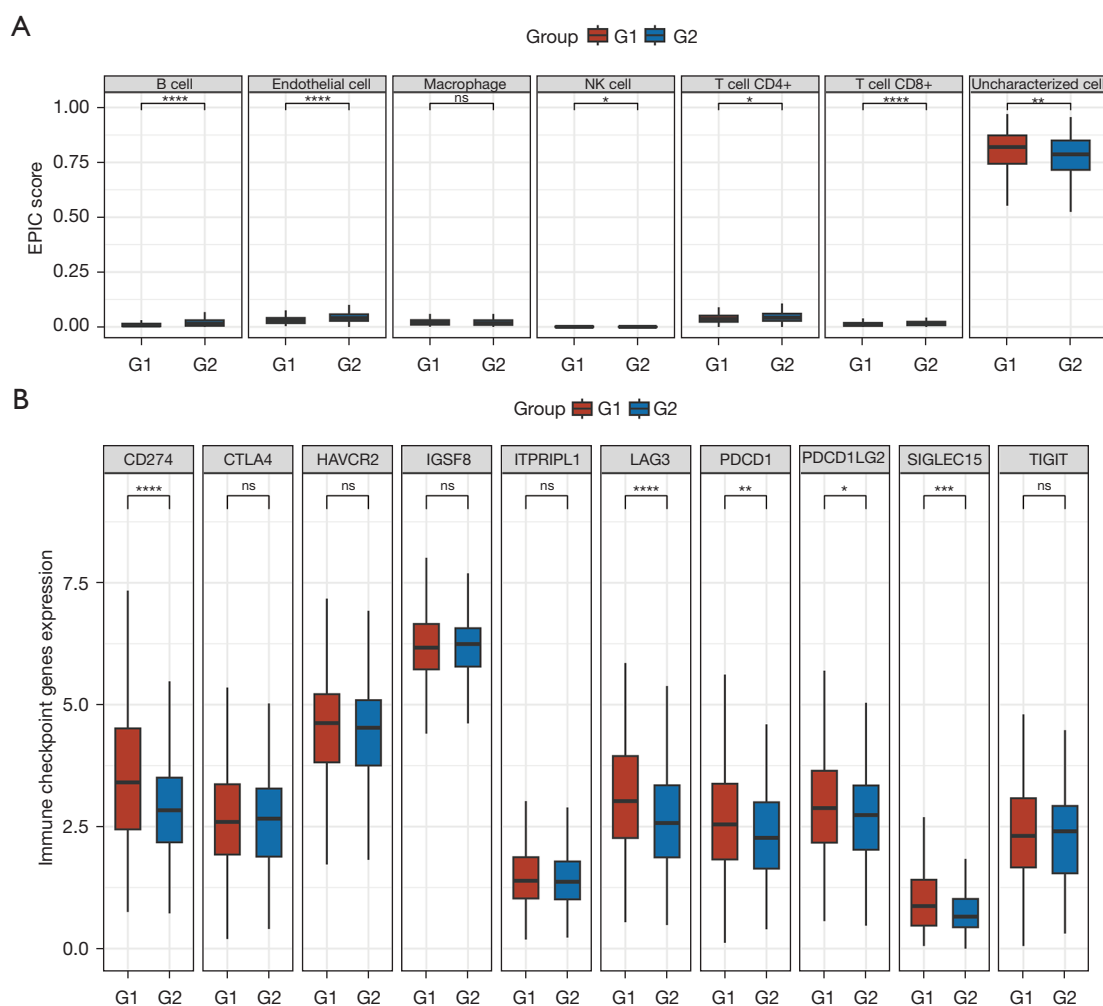
Figure 4 Immune activity in two DNA replication-related groups in LUAD. (A) Enrichment scores comparing seven immune cell types across two DNA replication–related clusters in LUAD. (B) Gene expression levels of immune checkpoint inhibitors between the two clusters in LUAD. *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001; ns, non-significant. EPIC, estimating the proportion of immune and cancer cells; LUAD, lung adenocarcinoma.

Immune activity between two DNA replication-related groups in LUAD

Numerous studies have reported a significant link between DNA replication and immune activity across various cancers (6-8). Initially, a Spearman correlation analysis was conducted to assess the relationship between the expression levels of DNA replication-related genes and immune scores. Several DNA replication-related genes (e.g., *FEN1*, *MCM5*, *POLD2*, *MCM4*, *MCM6*, *SSBP1*, *POLE2*, *RFC2*, *MCM2*, *PCNA*, *POLA2*, *MCM7*, *RFC3*, *POLE4*, and *RPA3*) were found to be strongly correlated with various immune cell types. We then compared the immune activity between the

two DNA replication-related clusters in LUAD patients. The boxplots revealed significant differences in the immune cells, including the B cells, endothelial cells, natural killer (NK) cells, cluster of differentiation (CD)4+ T cells, and CD8+ T cells, between the G1 LUAD samples and G2 LUAD samples (*Figure 4A*).

Additionally, the boxplots showed that 5 of the 10 immune checkpoint inhibitor (ICI)-related genes (i.e., *CD274*, *LAG3*, *PDCD1*, *PDCD1LG2*, and *SIGLEC15*) were more highly expressed in the G1 LUAD samples than in the G2 LUAD samples (*Figure 4B*). These findings suggest a strong association between DNA replication processes and immune activity.

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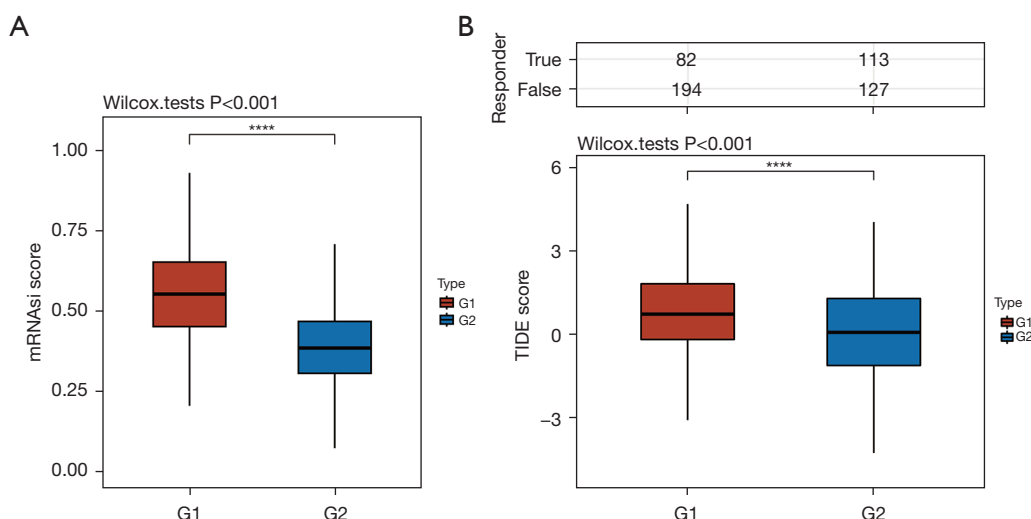
Figure 5 Tumor stemness in the two subtypes of LUAD. (A) Cancer stem cell scores for both LUAD subtypes and (B) TIDE scores. ****, P<0.0001. G1, Group 1; G2, Group 2; LUAD, lung adenocarcinoma; TIDE, tumor immune dysfunction and exclusion.

The tumor stemness of the two LUAD subtypes

Cancer stem cells (CSCs) are crucial for tumor initiation, recurrence, spread, and resistance to chemotherapy. There was a significant disparity in the CSC scores between the G1 and G2 risk groups (*Figure 5A*). Based on these findings, DNA replication-related classification could serve as a potential predictor of drug resistance.

A low survival rate was observed following immune checkpoint blockade (ICB) treatment in patients with elevated tumor immune dysfunction and exclusion (TIDE) scores. The G1 patients had higher TIDE scores than the G2 patients (*Figure 5B*), indicating G1 risk groups having an insufficient response to ICB therapy and poor prognosis.

Construction of a prognostic model based on DNA replication-related genes

Least absolute shrinkage and selection operator and Cox regression analyses were conducted, and 15 genes associated with DNA replication in LUAD were identified. A gene signature comprising these 15 genes was created based on the optimal lambda value (*Figure 6A*). Using this gene signature, TCGA-LUAD patients were stratified into high- and low-risk groups (*Figure 6B*). The survival analysis indicated that patients classified as low-risk had better OS than those classified as high-risk (HR: 1.833, 95% CI: 1.361–2.468, P<0.001; *Figure 6B*). The accuracy of the prognostic model was evaluated using a receiver operating

characteristic curve (ROC), resulting in areas under the curve (AUC) of 0.629 for 1 year, 0.634 for 3 years, and 0.582 for 5 years (*Figure 6C*), confirming the prognostic potential of the model.

Discussion

In this study, a total of 2,412 prognostic genes were obtained, and the DNA replication-related pathways closely associated with LUAD were identified by a KEGG enrichment analysis. The patients from the TCGA-LUAD cohort were divided into high- and low-risk groups based on the 15 DNA replication-related genes (i.e., *FEN1*, *MCM5*, *POLD2*, *MCM4*, *MCM6*, *SSBP1*, *POLE2*, *RFC2*, *MCM2*, *PCNA*, *POLA2*, *MCM7*, *RFC3*, *POLE4*, and *RPA3*). The upregulated genes were mainly related to the hallmarks of cancer (e.g., chromosome segregation, DNA replication, the cell cycle checkpoint, and DNA helicase activity), while the downregulated genes were mainly related to leukocyte activation involved in inflammatory macrophage activation, and passive transmembrane transporter activity. The immune cells, including the B cells, endothelial cells, NK cells, CD4+ T cells, and CD8+ T cells, in the G1 LUAD samples were clearly different from those in the G2 LUAD samples, and five of the 10 ICI-related genes were of a higher expression level in the G1 LUAD samples than the G2 LUAD samples. The tumor stemness of the two LUAD subtypes differed significantly. Furthermore, a six-gene (*FEN1*, *MCM5*, *POLD2*, *MCM4*, *SSBP1*, and *POLE4*)
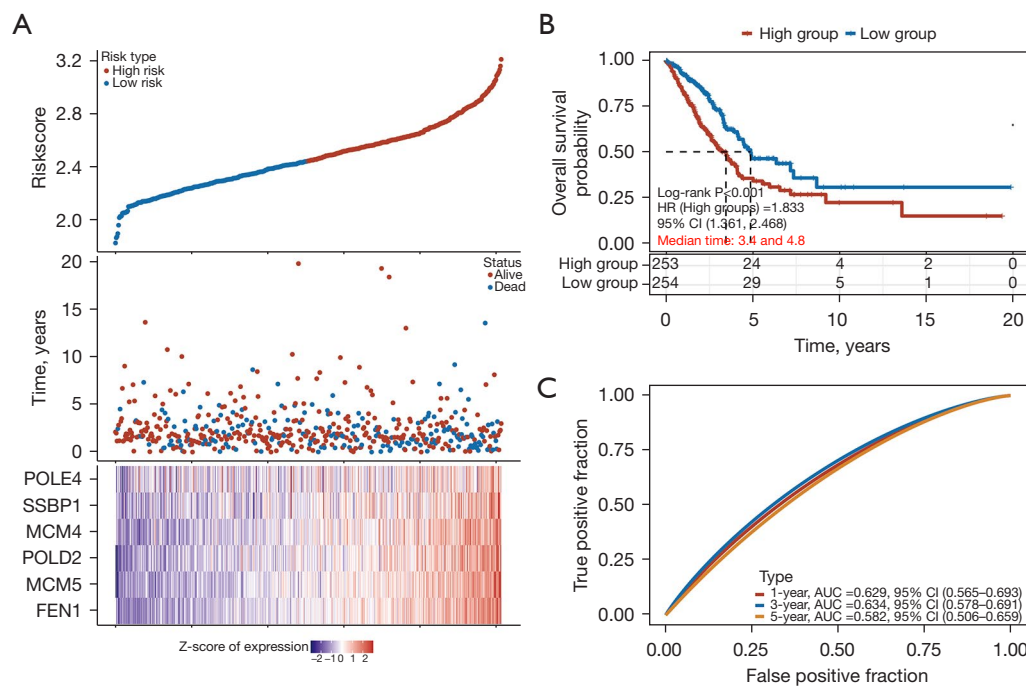
Figure 6 Development of a prognostic model based on DNA replication-related genes. (A) TCGA-LUSC patients were categorized based on the median risk score. Levels of 13 DNA replication-related genes are displayed in red for the high-risk group and blue for the low-risk group. (B) Survival analysis comparing the high-risk group to the low-risk group in TCGA-LUAD cohort. (C) Areas under the curve illustrate the prognostic model's accuracy in TCGA-LUAD cohort. AUC, area under the curve; CI, confidence interval; HR, hazard ratio; LUAD, lung adenocarcinoma; TCGA, The Cancer Genome Atlas.

prognostic model was constructed to predict the prognosis of LUAD patients.

We conducted a systematic analysis to investigate the role of DNA replication in LUAD. DNA replication, which causes genomic instability, is the most vulnerable cellular process in cancer development (7). When DNA damage is high, it can cause replication stress, which causes genome instability that has previously been found to be associated with pre-cancerous and cancerous cells (7). Using a novel and promising therapeutic strategy, cancer cells can be altered to integrate damaged dNTPs. T cancer cells can be selectively killed by targeting NUDT1 (also known as MTH1), a protein that prevents the misincorporation of oxidized dNTPs during replication (7-9). Understanding the molecular mechanisms underlying replication stress is essential in understanding tumorigenesis. However, the role of DNA replication in LUAD requires further study.

A positive correlation was found between DNA replication-related gene expression and prognosis among LUAD patients, and a six-gene (*FEN1*, *MCM5*, *POLD2*, *MCM4*, *SSBP1*, and *POLE4*) prognostic model was

constructed to predict the prognosis of LUAD patients. A previous study reported that flap endonuclease 1 (*FEN1*) promotes tumor progression and confers resistance to cisplatin in non-small cell lung cancer cells (10). HDAC1-mediated malignant progression of lung cancer is aggravated by minichromosome maintenance complex component 5 (MCM5) (11). Research has shown that DNA polymerase delta subunit 2 (POLD2) promotes the proliferation of triple-negative breast cancer by activating E2F1 (12). Minichromosome maintenance complex component 4 (MCM4) acts as a biomarker for LUAD prognosis (13). Mitochondrial single-stranded DNA binding protein (SSBP1) downregulation increases radiosensitivity in non-small cell lung cancer cells (14). DNA polymerase epsilon subunit 4 (POLE4) is a chaperone that maintains DNA replication integrity by co-chaperoning histone H3 and H4 (15).

Also, the interplay between DNA replication and immune checkpoint regulation is particularly relevant in the context of cancer. Tumor cells often exploit DNA replication stress to evade immune detection, leading to poor prognoses and treatment outcomes. For example,

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cancer stem cells (CSCs) can exhibit increased DNA repair capabilities and immune evasion, which contribute to their radioresistance and therapeutic failure (16). Moreover, the accumulation of cytosolic DNA as a result of replication stress can trigger innate immune responses, highlighting a complex relationship in which DNA replication not only influences immune cell activity but also shapes the immune landscape within tumors (17). Thus, investigating the effects of DNA replication on immune cell function and checkpoint regulation is crucial for advancing our understanding of immune responses in health and disease, particularly in cancer immunotherapy (18,19).

This study highlights the significance of a comprehensive bioinformatics analysis in understanding LUAD. By identifying key genes associated with prognosis, the developed six-gene prognostic model can be a valuable tool for clinicians. It has the potential to enhance patient stratification and inform treatment strategies, ultimately aiming to improve outcomes for LUAD patients. Focusing on DNA replication-related genes as prognostic markers indeed represents a novel and insightful approach to LUAD research. This emphasis addresses the critical need for improved patient stratification, which can significantly enhance personalized treatment strategies.

There are some limitations in this study. Compared to a previous study, a 2- transcription factor signature (1-year AUC=0.73, 2-year AUC=0.60 and 3-year AUC=0.61) based on the TCGA database was identified (20). In Yang *et al.* research, fatty acid synthesis and metabolism were used to construct the prognosis model for hepatocellular carcinoma, and the ROC curves indicated that the AUC for the high-risk group exceeded 0.7 at the 1-, 3-, and 5-year marks (21). Moreover, further *in vitro* and *in vivo* research needs to be conducted to investigate the specific role of DNA replication in LUAD. A prospective study may uncover the mechanisms by which DNA replication-related genes contribute to LUAD progression, which could provide new strategies for the treatment of LUAD.

Conclusions

There is a close relationship between the DNA replication-related genes and the tumor classification of LUAD patients. Our innovative signature incorporating DNA replication-related genes was found to be a good prognostic predictor of LUAD. Our findings may provide novel insights into the diagnosis and treatment of LUAD.

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