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Research Article

BIRC5 Expression Correlated with Immunosuppressive Phenotype and Predicted Inferior Response to Immunotherapy in Lung Adenocarcinoma

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Abstract

Background: Considering the pivotal role of *BIRC5* in tumorigenesis, recurrence, and chemoresistance, this study aimed to investigate its impact on the clinical and tumor microenvironmental features of Lung Adenocarcinoma (LUAD), together with its predictive and prognostic values.

Methods: Clinical and transcriptomic data of 535 LUAD samples, 59 normal lung, and 54 patients with Non-Small Cell Lung Cancer (NSCLC) received Immune Checkpoint Blockades (ICB) were analyzed. Deconvolution analysis was conducted to uncover the relationship between tumor microenvironmental features and *BIRC5* expression level. The predictive and prognostic values of *BIRC5* was also evaluated with Log-rank test and Cox regression analysis.

Results: LUAD had a significantly higher *BIRC5* expression level than normal lung tissues. The elevated *BIRC5* expression was markedly associated with unfavorable clinical outcomes. Transcriptomic and single-cell sequencing data analysis revealed that tumors with high *BIRC5* expression was correlated with multiple pathways' enrichment. Deconvolution analysis indicated a negative correlation between *BIRC5* expression and infiltration levels of CD8+ T cells, dendritic cells, and NK cells in LUAD, but a positive correlation was observed between *BIRC5* expression and regulatory T cells (Tregs) infiltrations. Importantly, NSCLC patients received ICB with high *BIRC5* expression had dramatically shorter progression-free (1.2 vs. 4.5 months; p=0.012) and overall survival (3.1 vs. 12.7 months; p=0.005) than those with low *BIRC5* expression.

Conclusion: These findings suggested that high *BIRC5* expression was associated with DNA damage/repair, cell invasion and proliferation related pathways enrichment and increased Tregs infiltration, which would result in inferior outcomes in NSCLC received ICB.

Keywords: Non-small cell lung cancer; Bioinformatics; *BIRC5*; TME; Immunotherapy

Introduction

Non-Small-Cell Lung Cancer (NSCLC) accounts for approximately 85% of all lung malignancies and is the leading cause of cancer-related deaths worldwide [1,2]. Among the histological subtypes, Lung Adenocarcinoma (LUAD) is most prevalent, leading to the majority of deaths attributable to lung cancers [3,4]. Despite huge treatment advancements in LUAD, including radiotherapy, molecular targeted therapy, and immunotherapy, the overall prognosis still remains unsatisfactory [5]. Immunotherapies, especially Immune Checkpoint Blockades (ICB) targeting programmed cell death protein 1 (PD-1), its ligand (PD-L1), or cytotoxic T lymphocyte antigen-4 (CTLA-4) significantly extend Overall Survival (OS) in patients with diverse types of cancer, including LUAD [6,7]. Nonetheless, a significant proportion of LUAD patients treated with ICB failed to achieve an objective response, highlighting the need for the identification of predictive biomarkers for selection of patients who may benefit from this treatment [8]. While ICB has shown to be an effective oncologic treatment for LUAD [9], the response to ICB can be affected by unique genomic and immunological landscapes [10]. Therefore, understanding correlates between specific gene expression and tumor immune microenvironmental features could help the development of robust biomarkers for immunotherapy and enhance the clinical response and expand the benefit population [11,12]. *BIRC5*, also known as survivin, is the smallest but functionally most complex member of the Inhibitor of Apoptosis Protein (IAP) family [13]. *BIRC5* plays a pivotal role in shielding cells from both intrinsic and extrinsic apoptotic pathways, primarily by indirectly inhibiting caspase 9 activation and physically preventing direct interactions with apoptosis-promoting molecules [14]. Functionally, *BIRC5* promotes malignant cell development by stabilizing the mitotic apparatus, maintaining proper chromosome segregation, and preserving microtubule integrity, facilitating safe and efficient cell division [15]. Hypoxia induces *BIRC5* expression, promoting angiogenesis and tightly linked to cell proliferation. *BIRC5* is highly expressed in most human cancers and strongly correlated

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with tumor growth, recurrence, chemotherapy resistance, and poor prognosis. However, a comprehensive analysis of *BIRC5* expression in LUAD and its prognostic and predictive value for ICB treatment outcomes has not yet been investigated. Therefore, we conducted this analysis to characterize the *BIRC5* expression and its correlation with clinicopathological features in LUAD, together with its predictive and prognostic values, using data from The Cancer Genome Atlas (TCGA), as well as the protein expression from the Human Pathology Atlas (HPA) online database. To elucidate the relationship between tumor microenvironmental features and *BIRC5* expression, we analyze the bulk RNA and single-cell sequencing data to investigate the connections between *BIRC5* expression level and enriched biological pathways and the immune cells' infiltrations.

Materials and Methods

Patients and databases

Gene expression data of 535 LUAD and 59 normal lung tissues was downloaded from the TCGA database (https://www.cancer.gov/ccg/research/genome-sequencing/tcga), with an additional 57 LUAD and matched normal lung tissues categorized for analysis of clinicopathological features and prognostic values. Transcriptomic data in Transcripts Per Million (TPM) formats from TCGA and GTEx (https://gtexportal.org/home/) was processed uniformly to examine *BIRC5* expression through both two databases. In addition, *BIRC5* expression data from pan-cancer tissues and surrounding normal tissues was retrieved and evaluated using the Oncomine database (https://www.oncomine.org). The protein level of *BIRC5* in LUAD and normal lung tissues was evaluated by using the Human Protein Atlas (HPA: https://www.proteinatlas.org/) database.

BIRC5-related functional enrichment analysis

To evaluate *BIRC5*-binding proteins, we utilized the STRING website (https://string-db.org/) v11.0 (archived version) and established specific thresholds to identify the binding proteins, including a network type of complete network, active interaction sources from studies, and a minimum necessary interaction score of low confidence (0.150). We adjusted the significance of network edges to evidence and limited the maximum number of interactors displayed to 20, resulting in the identification of 20 experimentally confirmed proteins that bind to *BIRC5*. Additionally, we utilized GEPIA2 (http://gepia2.cancerpku.cn/#index) to evaluate genes with a similar expression pattern to *BIRC5* in LUAD and selected the top 100 candidate genes. Subsequently, on the DAVID website (https://david.ncifcrf.gov), we performed Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses of *BIRC5*, 20 *BIRC5*-binding proteins, and 100 candidate genes.

Single-cell sequencing data analysis

We utilized the CancerSEA database (http://biocc.hrbmu.edu.cn/CancerSEA/home.jsp), a specialized platform for single cell sequencing data analysis, to investigate the functional status of cancer cells in relation to *BIRC5* expression. Correlation analysis was conducted to explore the association between *BIRC5* expression and various tumor functional characteristics, and a heatmap was generated based on the results. Moreover, we generated t-distributed stochastic neighbor embedding (t-SNE) diagrams for each single cell from the CancerSEA website to further evaluate the relationship between *BIRC5* and tumor cell functionality.

Differential gene expression analysis

R package "edgeR" was utilized to determine Differentially Expressed Genes (DEGs) between high and low SIRPG expression groups. A cutoff gene expression fold change of >1.5 or <−1.5 and FDR q-value <0.05 was applied to select the most significant DEGs. We listed all of the DEGs by using volcano plot and the top 100 up-regulated genes in high versus low *BIRC5* expression group by using heatmap.

Pathway enrichment analysis

We applied three methods (GO, KEGG, GSEA) to perform the pathway enrichment analysis. The curated gene sets of reported signaling pathways (from the KEGG, Hallmark, PID, Reactome databases) were downloaded from the molecular signature database (http://software.broadinstitute.org/gsea/msigdb/index.jsp). R package "clusterProfiler" was used for GO term analysis, and GSEA software V.4.1.0 was utilized to study the relevant pathways between high and low SIRPG expression groups.

Infiltration of immune cells

To quantify the immune cell infiltration in tumor tissues, we employed single-sample Gene Set Enrichment Analysis (ssGSEA) method. The LUAD expression profile data was subjected to immunological datasets encompassing 24 categories of immunocytes, and the infiltration levels were quantified using the R package "GSVA" [16]. Furthermore, Spearman correlation and Wilcoxon rank sum test were implemented to evaluate the association between *BIRC5* and various immune cells as well as the relationship of immune cells with the two groups.

Public datasets of anti-PD-1/PD-L1 treated study

The clinical and bulk RNA-seq data of 54 NSCLC patients received PD-1/PD-L1 blockade monotherapy, along with response data available, were downloaded from previous publications [1,17,18]. The clinical responses were assessed by the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 guideline, including Complete Response (CR), Partial Response (PR), Stable Disease (SD) or Progressive Disease (PD). Responders were defined as patients received PD-1/PD-L1 blockade with CR or PR or SD. Non-responders were defined as patients received PD-1/PD-L1 blockade with PD. The definition of Progression-Free Survival (PFS) and Overall Survival (OS) was consistent with their corresponding published studies. Kaplan-Meier curves with two-sided log-rank tests and Cox proportional hazards model with calculated Hazard Ratios (HRs) and 95% Confidence Intervals (CIs) were used to determine the survival difference.

Statistical analysis

BIRC5 expression in normal lung and LUAD groups was analyzed using the Wilcoxon rank-sum test. Patients with LUAD were divided into two groups based on the median expression of *BIRC5*. The clinicopathological characteristics comparison between high and low *BIRC5* expression groups were analyzed using Wilcoxon rank sum or Kruskal-Wallis tests. Pearson correlation analysis were calculated to evaluate the relatedness of *BIRC5* expression and immune cells abundance, immune-related markers expression. Survival outcomes were measured with OS, Disease-Free Survival (DFS), or PFS according to the accessibility for each cohort. Prognostic and predictive values of *BIRC5* expression were analyzed by Kaplan-Meier method, log-rank test, and Cox proportional hazards regression analysis. To study

the predictive significance of differentially expressed genes, a Receiver Operating Characteristic (ROC) curve was developed using the "plotROC" package. The predictive value nomogram for LUAD patients was plotted using the R tool "rms." All statistical significance testing was two-sided and p values or FDR q values <0.05 were considered statistically significant. All tests were performed with the R environment v4.0 or GraphPad Prism version 6.0.

Results

Characterization of *BIRC5* expression in LUAD

Firstly, we analyzed the distinct expression levels of *BIRC5* between normal lung and LUAD tissues using transcriptomic datasets from the TCGA program. The detailed information of 535 LUAD patients and *BIRC5* expression were listed in Table 1. The expression level of *BIRC5* was significantly higher in LUAD tissues compared to normal lung samples (p<0.001; Figure 1A). In the paired tissue samples, LUAD also showed a significantly higher expression level of *BIRC5* than normal lung tissues (p<0.001; Figure 1B). To analyze the expression level of *BIRC5* in various cancers, we carried out a systemic analysis using transcriptomic datasets from the TCGA program and found that *BIRC5* was upregulated in most cancer types, including bladder urothelial carcinoma, bone cancer, cholangiocarcinoma, and glioblastoma multiforme (Figure 1C). Using HPA database, we evaluated the expression levels of *BIRC5* in LUAD and normal lung tissues. We observed that LUAD had higher protein levels of *BIRC5* than the matched normal lung tissues (Figure 1D). Moreover, tumor with pathological stage II-IV had significantly higher expression of *BIRC5* than those with pathological stage I (p<0.001) (Table 1 and Figures 1A-1D). (p<0.001; Figure 2A). We also found that increased T stage (p<0.001), N stage (p<0.001), smoking history (p< 0.05), and young age (p<0.05) were associated with elevated expression of *BIRC5* (Figures 2A-2F). To determine the prognostic value of *BIRC5* in LUAD, we examined the correlation between *BIRC5* expression and survival outcomes of LUAD patients. The high expression of *BIRC5* was correlated with markedly inferior OS (HR=1.79, p<0.001; Figure 3A), DFS (HR=1.98, p<0.001; Figure 3B), and PFS (HR=1.58, p=0.001; Figure 3C). In addition, the ROC analysis of LUAD to determine the predictive significance of *BIRC5* revealed an Area Under the Curve (AUC) value of 0.96 (Figure 3D) (Figures 3A-3D).

Analysis of *BIRC5* co-expression network and enriched pathway

We identified and experimentally validated the binding proteins of *BIRC5* using the STRING website to investigate the co-expression network and pathways enrichment of *BIRC5* in LUAD. The results showed the presence of 20 proteins, including MED1, MED6, MED18, MED30, MED31, LAMTOR5, H3F3A, H3F3B, AURKB, AURKC, HFE, INCENP, CDCA8, HIST3H3, CASP3, CASP7, CASP9, DIABLO, XIAP and BIRC2 binding to *BIRC5* (Figure 4A). Then, we extracted the 100 most closely linked genes to *BIRC5* from GEPIA2 database. GO and KEGG enrichment analyses were conducted on the 448 genes. GO enrichment analysis revealed that these genes were significantly associated with chromosome segregation, mitotic nuclear division, nuclear division, mitotic sister chromatid segregation, ATPase activity, microtubule motor activity, tubulin binding, microtubule binding, and other processes (Figures 4B-4D). In addition, we observed that *BIRC5* was implicated in tumorigenesis via cell cycle, pyrimidine metabolism, cellular senescence, DNA replication, p53 signaling pathway, and progesterone-mediated oocyte maturation (Figure 4E). Taken together, these results were consistent with the classical role of *BIRC5* in previous publications Figures 4A-4E.

Characteristic	Low expression of *BIRC5*	High expression of *BIRC5*	p-value
n	267	268	-
T stage, n (%)			0.001
T1	108 (20.3%)	67 (12.6%)	-
T2	124 (23.3%)	165 (31%)	-
T3	24 (4.5%)	25 (4.7%)	-
T4	10 (1.9%)	9 (1.7%)	-
N stage, n (%)			0.002
N0	190 (36.6%)	158 (30.4%)	-
N1	38 (7.3%)	57 (11%)	-
N2	27 (5.2%)	47 (9.1%)	-
N3	0 (0%)	2 (0.4%)	-
M stage, n (%)			0.149
M0	177 (45.9%)	184 (47.7%)	-
M1	8 (2.1%)	17 (4.4%)	-
Pathologic stage, n (%)			<0.001
Stage I	169 (32.1%)	125 (23.7%)	-
Stage II	53 (10.1%)	70 (13.3%)	-
Stage III	31 (5.9%)	53 (10.1%)	-
Stage IV	9 (1.7%)	17 (3.2%)	-
Primary therapy outcome, n (%)			0.005
PD	23 (5.2%)	48 (10.8%)	-
SD	22 (4.9%)	15 (3.4%)	-
PR	3 (0.7%)	3 (0.7%)	-
CR	179 (40.1%)	153 (34.3%)	-
Gender, n (%)			<0.001
Female	164 (30.7%)	122 (22.8%)	-

Male	103 (19.3%)	146 (27.3%)	-
Race, n (%)			0.817
Asian	3 (0.6%)	4 (0.9%)	-
Black or African American	30 (6.4%)	25 (5.3%)	-
Residual tumor, n (%)			0.185
R0	166 (44.6%)	189 (50.8%)	-
R1	7 (1.9%)	6 (1.6%)	-
R2	0 (0%)	4 (1.1%)	-
Anatomic neoplasm subdivision 2, n (%)			1
Central lung	28 (14.8%)	34 (18%)	-
Peripheral lung	56 (29.6%)	71 (37.6%)	-
Smoker, n (%)			0.078
No	45 (8.6%)	45 (8.6%)	-
Yes	215 (41.3%)	231 (44.3%)	-
Age, median (IQR)	67 (60,74)	65 (58,71)	0.015
Number pack years smoked, median (IQR)	30 (20,50)	40 (25,54)	0.007

Table 1: Baseline characteristics comparison between high and low *BIRC5* expression groups.

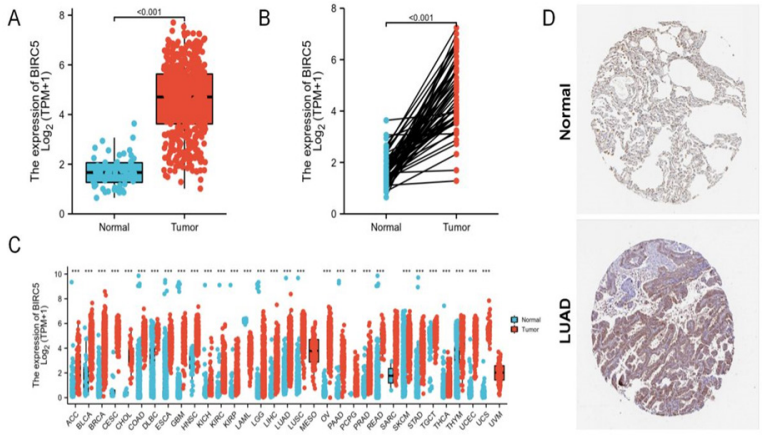

Figure 1: The expression level of *BIRC5*. (A) Comparison of *BIRC5* expression level between LUAD and normal lung tissues; (B) *BIRC5* expression level in 57 matched LUAD and normal lung tissues; (C) Pan-cancer analysis of *BIRC5* expression in various cancers and adjacent normal tissues; (D) Human Protein Atlas (HPA) database were applied to characterize the *BIRC5* protein expression. Note: **p<0.01; ***p<0.001.

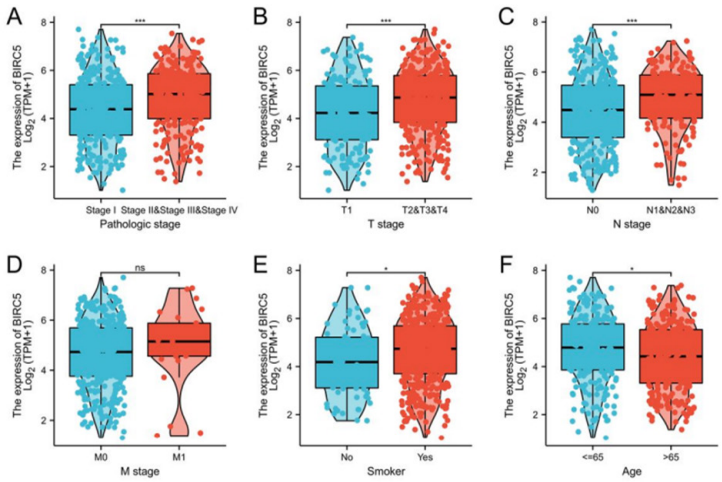

Figure 2: Correlation between *BIRC5* expression and clinicopathological features. (A) Clinical stage; (B) T stage; (C) N stage; (D) M stage; (E) Smoking history; (F) Age. Note: *p<0.05; **p<0.01; ***p<0.001.

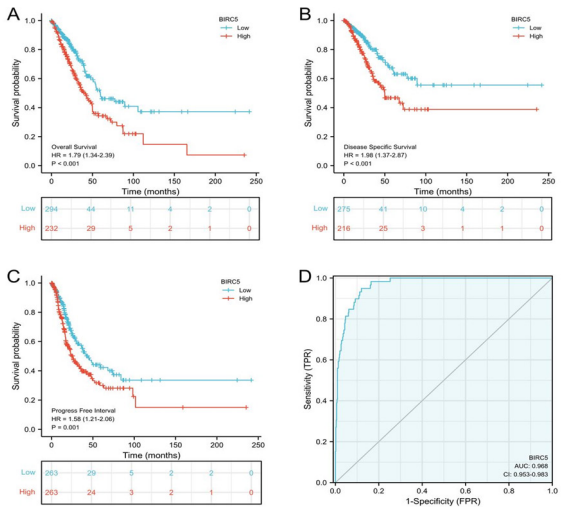

Figure 3: Survival analysis of clinical outcomes between patients with high and low *BIRC5* expression. (A) Kaplan-Meier plot of overall survival; (B) Kaplan-Meier plot of disease-specific survival; (C) Kaplan-Meier plot of progression-free survival; (D) ROC analysis of LUAD to determine the predictive significance of *BIRC5*.

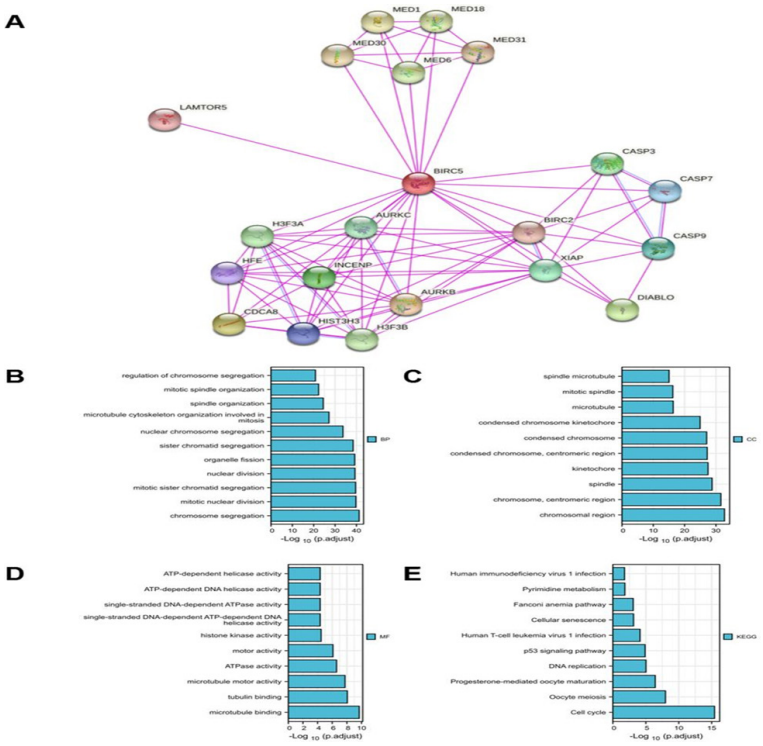

Figure 4: Co-expression network of *BIRC5* and enrichment pathway analysis. (A) The binding proteins of *BIRC5* by STRING website; (B-D) Gene Ontology (GO) enrichment analysis of biological process, cellular component, molecular function; (E) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways analysis based on *BIRC5* binding proteins and interactive genes.

Expression level of *BIRC5* in single cells and its effect on functional status

Single-cell transcriptomic sequencing is a critical tool for analyzing the complex landscape of tumor, immune, endothelial, and stromal cells in cancers. In our investigation of *BIRC5* expression and its association with tumor function in LUAD at the single-cell level, we utilized the CancerSEA website and found that *BIRC5* expression was significantly correlated with the critical cellular processes such as cell cycle, DNA damage, DNA repair, hypoxia, inflammation, and proliferation (Figure 5A). Our analysis further revealed that *BIRC5* expression was associated with cell cycle progression, DNA damage response, proliferation, invasion, Epithelial Mesenchymal Transition (EMT), and inflammation in LUAD (Figure 5B). We also visualized *BIRC5* expression profiles in single cells of LUAD using t-SNE diagrams (Figure 5C). These findings suggest that *BIRC5* may exert a crucial function in the development of LUAD by modulating key cellular processes such as the cell cycle, DNA damage/repair, hypoxia, inflammation, and proliferation (Figures 5A-5C).

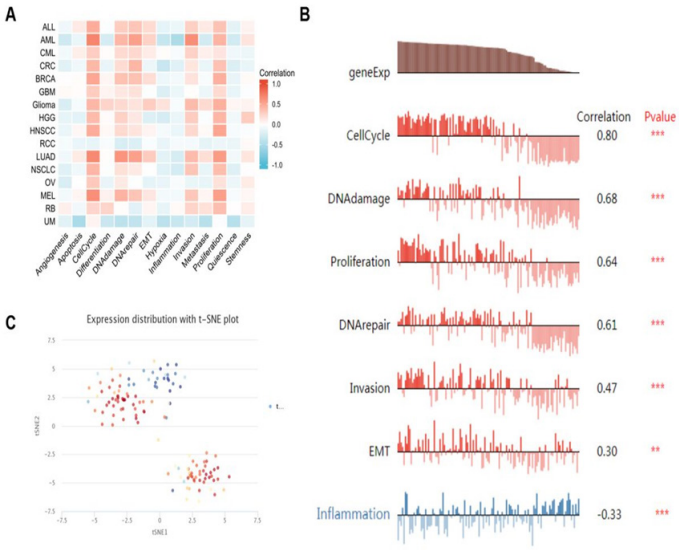

Figure 5: Expression pattern of *BIRC5* in single cells and its correlation with tumor functional status. (A) Heatmap showed the correlation between *BIRC5* expression and different tumor functional status based on CancerSEA database; (B) Correlation between *BIRC5* expression and seven significantly different functional states based on CancerSEA database; (C) t-SNE diagram of *BIRC5* expression profiles in single cells.

Univariate and multivariate analyses

Having noticed the negative association between high *BIRC5* expression and clinical outcomes of LUAD patients, we then conducted the univariate and multivariate analyses by adjusting the common clinicopathological parameters. The results showed that T, N, and M stages, pathological stage, residual tumor, and tumor status were significantly associated with OS in univariate analyses (Supplemental Table S1). High *BIRC5* expression was also markedly associated with poor OS (HR=1.75, 95%CI 1.30–2.36, p<0.001). In multivariate analyses, high *BIRC5* expression was found to be independently associated with shorter OS in multivariate analysis (HR=1.44, 95% CI 1.01–2.28, p=0.043). We performed a subgroup analysis to investigate the impact of *BIRC5* expression on OS based on age, sex, and anatomic neoplasm subdivision risk variables. In these subgroups stratified by age, gender, and anatomic neoplasm classification, we observed that elevated *BIRC5* expression was significantly associated with worse survival outcomes (Supplemental Figure 1).The aforementioned results demonstrate a strong association between the *BIRC5* expression levels and clinical outcomes in LUAD patients. Accordingly, we developed a predictive nomogram based on these variables to estimate individual survival probabilities (Supplemental Figure 2A). The calibration curve of the predictive model revealed that the 1-3 years and 5-year survival estimates closely aligned with the ideal line (Supplemental Figure 2B). These findings demonstrate that *BIRC5* expression-based model could provide accurate estimate of overall prognosis.

Enriched pathways in tumor with high *BIRC5* expression

To identify the different pathways enrichment between high and low *BIRC5* expression groups, we performed GSEA using bulk RNA sequencing data from 535 LUAD patients. The results revealed that tumors with high *BIRC5* expression was associated with several enriched pathways including mRNA splicing, DNA repair, and translation (Supplemental Figure 3). Single-cell sequencing data analysis also uncovered a potential mechanistic link between *BIRC5* expression and cell cycle, DNA damage/repair, hypoxia, inflammation, and cell proliferation.

Immune infiltration analysis

To determine the immune profiles of tumors with distinct *BIRC5* expression levels, we deconvoluted the bulk RNA sequencing data of 535 LUAD patients to depict immune infiltration landscape. The deconvolution analysis indicated a negative correlation between *BIRC5* expression and infiltration levels of several immunosupportive cells including CD8 + T cells, T helper 17 cells, B cells, macrophages, dendritic cells, and NK cells in LUAD, but a positive correlation was observed between *BIRC5* expression and regulatory T cells (Tregs) infiltration levels (Figure 6). These results suggest that tumors with high *BIRC5* expression would possess a cold tumor immune microenvironment.

Predictive value of *BIRC5* expression in LUAD received ICB

Generally, tumors with high immunosuppressive cells infiltration are unlikely to respond to immunotherapy. Having noticed LUAD with high *BIRC5* expression had elevated Tregs infiltration, we next hypothesized that NSCLC patients whose pre-treatment tumors with high *BIRC5* expression may show poor response to PD-1/PD-L1 blockade. To determine the impact of *BIRC5* expression on clinical outcomes in patients treated with PD-1/PD-L1 blockade monotherapy, we conducted an integrated analyses of the transcriptomic and treatment outcome data of patients with advanced NSCLC receiving PD-1/PD-L1 blockade monotherapy. Three publications with 54 NSCLC patients had available transcriptomic and response data. Firstly, we observed that responders had significantly lower *BIRC5* expression level than non-responders (p=0.029, Figure 7A). Patients with high *BIRC5* expression (≥ median level) showed a markedly lower response rate than those with low *BIRC5* expression (<median level) (ORR 19.0% vs. 41.1%; p=0.024, Figure 7B). Importantly, patients with high *BIRC5* expression had dramatically longer PFS than those with low *BIRC5* expression (median PFS 1.2 vs. 4.5 months; p=0.012, Figure 7C). OS was also shorter in high *BIRC5* expression group than in low group (median OS 3.1 vs. 12.7 months; p=0.005, Figure 7D). We did not perform the multivariate analysis because the clinicopathological data of these studies were unavailable (Figures 7A-7D).

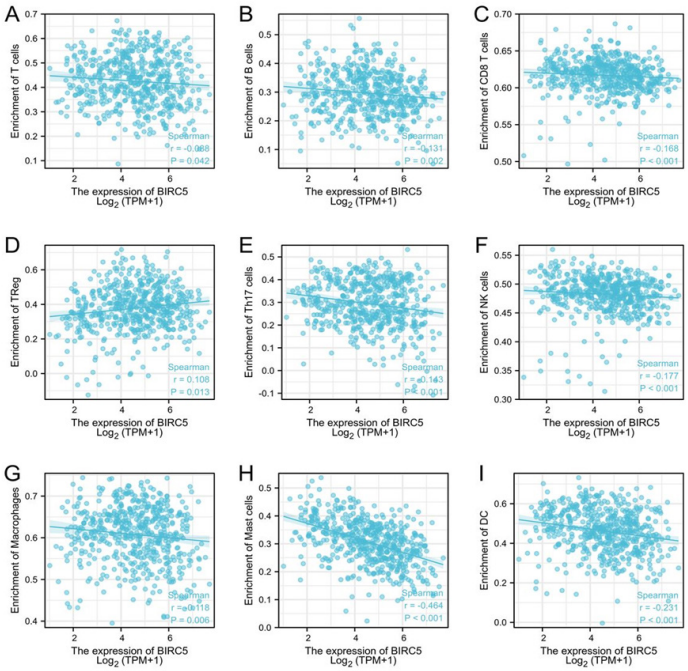

Figure 6: The correlation between immune cell infiltrating and *BIRC5* expression level in LUAD1.

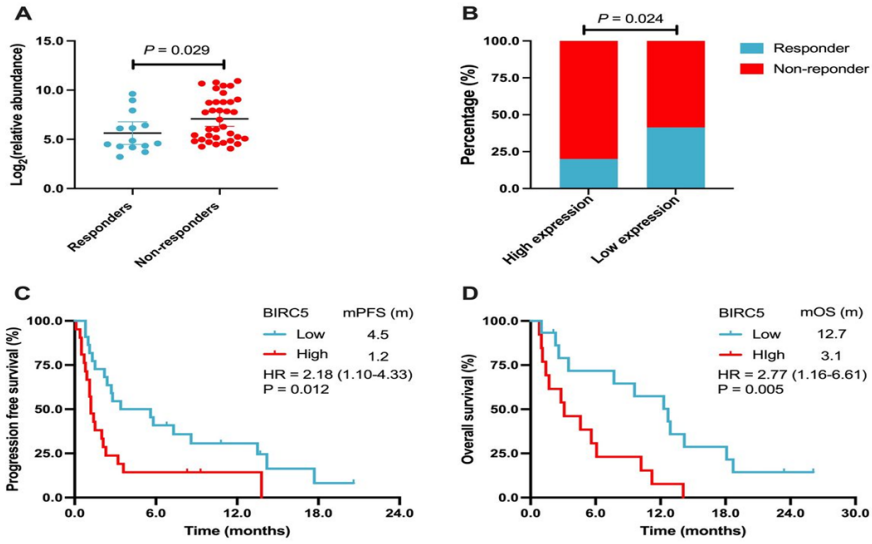

Figure 7: Predictive value of *BIRC5* expression in LUAD received Immune Checkpoint Blockade (ICB). (A) Responders had significantly lower *BIRC5* expression level than non-responders; (B) Patients with high *BIRC5* expression (≥ median level) showed a markedly decreased response rate than those with low *BIRC5* expression (<median level); (C) Survival analysis of *BIRC5* expression in NSCLC patients with PD-1 blockade for PFS; (D) Survival analysis of *BIRC5* expression in NSCLC patients with PD-1 blockade for OS.

Discussion

In this study, we integrated the clinical and transcriptomic data of 535 LUAD samples, 59 normal lung, and 54 patients with NSCLC treated with ICB from online database and found that LUAD had a significantly higher expression level of *BIRC5* than normal lung tissues. The elevated *BIRC5* expression was markedly associated with unfavorable clinical outcomes. Transcriptomic and single-cell sequencing data analysis revealed that tumors with high *BIRC5* expression was associated with enrichment of cancer cell invasion and proliferation related pathways including cell cycle, DNA damage/repair, hypoxia, inflammation, and cell proliferation. The deconvolution analysis indicated a negative correlation between *BIRC5* expression and infiltration levels of CD8+ T cells, T helper 17 cells, B cells, macrophages, dendritic cells, and NK cells in LUAD, but a positive correlation was observed between *BIRC5* expression and Tregs infiltrations. Importantly, NSCLC patients received PD-1/PD-L1 inhibitors with baseline high *BIRC5* expression had significantly shorter PFS and OS than those with low *BIRC5* expression. Apoptosis is a crucial process that maintains normal cellular function [19]. Normally, apoptosis helps eliminate damaged

DNA and cells with irregular cell cycles [20]. However, in malignant cells, the apoptotic mechanism is disrupted and the body is unable to effectively eliminate these cells, leading to tumorigenesis and treatment resistance [21]. The IAP protein family is a highly conserved group of antiapoptotic factors that primarily inhibit apoptosis by suppressing caspase activity and regulating NF-κB signaling [22]. *BIRC5*, the smallest member of the IAP family and located on chromosome 17q25, was isolated from the human genome library in 1997(23). *BIRC5* has various biological functions, including the inhibition of apoptosis by suppressing caspase-3 and caspase-7 activity in direct or indirect ways, promoting cell mitosis by assisting in chromosome division and microtubule movement, angiogenesis, and serving as a biomarker for tumor diagnosis, treatment, and prognosis prediction [24]. Thus, inhibition of *BIRC5* activity may help to inhibit the development and spread of tumor cells, making it a promising target for cancer therapy. Numerous studies have provided evidence for the critical role of *BIRC5* in regulating cell cycle, proliferation, progression, and angiogenesis in cancer cells [25]. Cytokines in lymphocytes have been shown to control the expression of *BIRC5*, which is essential for the growth and survival of hematopoietic cells. In the context of human cancers, *BIRC5* has been reported to inhibit cell apoptosis while stimulating cell proliferation [26,27]. Previous studies have revealed that *BIRC5* exerts its anti-apoptotic effects through the suppression of both caspase-dependent and caspase-independent apoptotic pathways, in addition to promoting cell growth and development. In this study, we integrated the bulk and single-cell RNA sequencing data and observed that tumors with high *BIRC5* expression were correlated with cell cycle, DNA damage/repair, hypoxia, inflammation, and cell proliferation pathways enrichment. Moreover, LUAD patients with high *BIRC5* expression had unfavorable clinical outcomes. Collectively, these findings revealed that *BIRC5* may play a pivotal role in tumorigenesis and prognosis in LUAD. Hypoxia, a characteristic feature of the tumor microenvironment of advanced solid tumors, has been extensively studied [28]. As an important stressor, hypoxia can regulate the expression of a number of genes within tumor cells, allowing the cells to adapt to their hypoxic surroundings [29]. Among the genes affected by hypoxia, Vascular Endothelial Growth Factor (VEGF) and related factors have been shown to induce tumor recurrence and the production of a large number of vascular growth factors [30]. Furthermore, hypoxia has been found to upregulate *BIRC5* expression, which in turn enhance angiogenesis and is closely linked to cell proliferation [31]. The hypoxic tumor microenvironment is closely associated with poor outcomes and resistance to immunotherapy in various cancers [32]. Targeting hypoxic cells can be achieved by suppressing Hypoxia-Inducible Factors (HIFs), which are essential for their survival. In this context, the development of a *BIRC5* inhibitor that targets HIFs may represent a promising therapeutic approach for patients with advanced lung cancer and those who are resistant to immune-based therapies.

In recent years, the role of immune cell infiltration in cancer development has received considerable attention [33]. The immune cell infiltrate composition and immune gene signatures have been shown to be the robust predictors of clinical prognosis in several cancers. The presence of CD8 and memory T cells, as well as Th1-biased gene signatures, is associated with a better prognosis, while the presence of M2-like macrophages is associated with a worse prognosis [34,35]. In our study, we observed an inverse correlation between *BIRC5* expression in LUAD and immune cells such as CD8+ T cells, macrophages, B cells, T helper 17 cells, DCs, and NK cells. Moreover, we found that *BIRC5* expression was positively correlated with Tregs, which have been linked to poor prognosis in cancer patients due to the suppressive anti-tumor immune effect of Tregs. These results suggest that *BIRC5* may play a significant role in regulating the tumor immune microenvironment. However, the precise mechanism by which *BIRC5* influences the tumor immune microenvironment and the tumorigenesis of LUAD remains unknown. To unravel the detailed mechanism of *BIRC5* mediating CD8+ T cell phenotype and function transition and regulating Tregs infiltration is worthwhile for the development of novel immunotherapeutic targets and combination therapeutic strategies. Immunotherapy has been emerged as a successful cancer therapy. However, not all patients respond to immunotherapy. Our findings showed that in LUAD patients treated with PD-1/PD-L1 inhibitors, those with low *BIRC5* expression had a significantly higher ORR, PFS and OS than those with high *BIRC5* expression. These results suggested that *BIRC5* expression might represent as a potential biomarker to predict the response to immunotherapy in LUAD. Further investigations with large sample size are needed to validate its predictive value.

Conclusion

In conclusion, the current study indicated that high *BIRC5* expression was associated with enrichment of DNA damage/repair, cancer cell invasion and proliferation related pathways, increased Tregs infiltration, and unfavorable response to PD-1/PD-L1 blockade in patients with LUAD, suggesting that it would be a promising predictive biomarker for PD-1/PD-L1 blockade and novel potential immunotherapeutic target in LUAD.

Declarations

Acknowledgements

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Authors' Contributions

All authors contributed to the study conception and design. Xiaohui Chen, Jia Yu, Shengxiang Ren raised the idea. Material preparation, data collection and analysis were performed by Shuo Yang, Xiaozhen Liu, Shiqi Mao, Chuchu Shao, Xuefei Li and Chao Zhao. The first draft of the manuscript was written by Shuo Yang, Xiaozhen Liu, Yan Wang, Qiyu Fang and Bin Chen. Fengying Wu and Xiaoxia Chen revised the manuscript. All authors read and approved the final manuscript.

Ethics statement

This study was reviewed and approved by Institutional Review Board of our center. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors on request.

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