Letter to the Editor

Role of Prognostic Marker *PRR11* in Immune Infiltration for Facilitating Lung Adenocarcinoma Progression*

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The *PRR11* gene (Proline Rich 11) has been implicated in lung cancer; however, relationship between *PRR11* and immune infiltration is not clearly understood. In this study, we used The Cancer Genome Atlas (TCGA) data to analyze the lung adenocarcinoma patients; *PRR11* gene expression, clinicopathological findings, enrichment, and immune infiltration were also studied. *PRR11* immune response expression assays in lung adenocarcinoma (LUAD) were performed using TIMER, and statistical analysis and visualization were conducted using R software. All data were verified using Gene Expression Profiling Interactive Analysis (GEPIA), and the Human Protein Atlas (HPA). We found that *PRR11* was an important prognostic factor in patients with LUAD. *PRR11* expression was correlated with tumor stage and progression. Gene Set Enrichment Analysis (GSEA) showed that *PRR11* was enriched in the cell cycle regulatory pathways. Immune infiltration analysis revealed that the number of T helper 2 (Th2) cells increased when *PRR11* was overexpressed. These results confirm the role of *PRR11* as a prognostic marker of lung adenocarcinoma by controlling the cell cycle and influencing the immune system to facilitate lung cancer progression.

Key words: Bioinformatics; Lung adenocarcinoma; *PRR11*; Cell cycle; Th2 cell

Lung cancer is associated with high rates of morbidity and mortality worldwide[1], with non-small cell lung cancer (NSCLC) most commonly presenting as lung adenocarcinoma (LUAD). Due to the absence of early stage symptoms in patients diagnosed with LUAD, the disease often progresses to advanced stages before the first diagnosis; consequently, treatment effectiveness is severely limited[2]. LUAD is currently treated with surgery and a variety of other treatments; however, patients with lung cancer are at risk of mortality due to local recurrences and distant metastases. The metastatic processes of LUAD includes invasion, circulatory dissemination, distant cloning, and angiogenesis. Understanding the pathogenesis, progression, and molecular mechanisms of drug resistance in LUAD is key to precision therapy. Biomarker screening is vital for accurate diagnosis and effective treatment of LUAD. Previous studies of LUAD have found potential epidermal growth factor receptor (EGFR) mutations and anaplastic lymphoma kinase (ALK) fusions or rearrangements in patients. In clinical practice, only a few patients are eligible for targeted therapies; therefore, treatment and prognosis of LUAD are greatly influenced by validating clinically useful targets.

Recent research has identified *PRR11* as a gene on chromosome 17q22 that performs specific functions in lung cancer development and cell cycle regulation. *PRR11* contains a zinc finger domain and two proline-rich regions; double-stranded DNA binds to zinc regulators that regulate gene transcription. *PRR11* is widely distributed throughout the cytoplasm, cytoskeleton, and nucleus[3]. The biological structure of the *PRR11* protein is comprised of three main parts: a binary nuclear localization signal, proline-enriched region, and zinc finger domain including a proline-enriched motif associated with SH3, which regulates protein-protein interactions, participates in intracellular signal transduction, and triggers tumor malignant biological behavior.

Significant evidence suggests that *PRR11* is highly expressed in tumors associated with gastric cancer,

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breast cancer, and hilar cholangiocarcinoma, and plays a critical role in maintaining malignant phenotypes in cancer cells. *PRR11* knockdown in lung cancer cells inhibits cell proliferation, cell cycle progression, migration, invasion, and colony formation *in vitro*. Although the involvement of *PRR11* in lung cancer has been established, the relationship between *PRR11* and immune infiltration has not comprehensively explored. Thus, the purpose of this study was to classify lung adenocarcinoma patients using bioinformatics methods to examine the relationship between *PRR11* and immune infiltration. The findings of this study will aid in the use of *PRR11* as a potential marker for the diagnosis, treatment, and prognosis of LUAD.

We obtained gene expression, clinical, and immune infiltration data from TCGA database (https://gdc.nci.nih.gov). The lung adenocarcinoma transcriptome dataset included 539 tumor samples and 59 adjacent tissue samples. Gene enrichment analysis (GSEA) was performed using normalized RNA-Seq data from TCGA. To investigate the potential biological functions of *PRR11*, GSEA was used to identify Gene Ontology (GO) functions and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. To be considered statistically significant, enrichment results must meet two conditions: false discovery rate (FDR) < 0.05 and $P < 0.05$. Using the Gene Expression Profiling Interactive Analysis (GEPIA) online database (http://gepia.cancerpku.cn/), *PRR11* expression in lung adenocarcinomas was compared to that in normal tissues and the relationship between overall survival (OS) and *PRR11* expression in LUAD was determined. The Human Protein Atlas Database (HPA) (http://proteinatlas.org) was used to compare the protein expression of *PRR11* in lung adenocarcinoma and normal lung tissues. The TIMER (https://cistrome.shinyapps.io/timer/) analysis tool was used for systematic analysis of immune infiltrates in various types of cancer. Evaluation of the potential relationship between *PRR11* expression and tumor-infiltrating immune cells was performed using the TIMER correlation module. A literature search was used to identify 24 tumor-infiltrating immune cells and single-sample gene set enrichment analysis (ssGSEA) was carried out using the "GSVA" R package to determine their relationship with *PRR11* expression in LUAD. R software v.4.2.1 (R Core Team 2022) was used for all statistical analyses. Univariate and Multivariate Cox models were used to calculate 95% *CI*s and *HR*s. Univariate survival analysis was performed to examine the relationships between several clinical characteristics and survival. The impact of *PRR11* expression and other pathological and clinical factors on overall survival were evaluated based on the results of Multivariate Cox analysis. Logistic Regression analysis was used to analyze the association between the clinical characteristics and *PRR11* expression.

Bioinformatics analysis identified high *PRR11* expression in most of cancers (Figure 1A). *PRR11* mRNA expression levels in normal tissues and lung adenocarcinomas were studied using TCGA database. Differential analysis was performed on data from 59 normal and 539 tumor tissue samples. *PRR11* expression was significantly higher in tumor tissue samples than that in normal samples ($P < 0.001$, Figure 1B). As shown in Supplementary Table S1 (available in www.besjournal.com), the association between *PRR11* expression and several characteristic variables of OS in patients with lung adenocarcinoma was examined using Cox regression analysis. Pathological stage ($P < 0.001$), tumor TNM stage ($P < 0.01$), and *PRR11* expression ($P < 0.001$) were significantly associated with OS. Multivariate analysis results are shown in Figure 1C; *PRR11* expression ($P = 0.008$) played a significant role in prognosis (Supplementary Table S2, available in www.besjournal.com). Patient *PRR11* expression distributions and survival statuses are shown in Figure 1D. The AUC value of *PRR11* expression on the ROC curve was 0.612 (Figure 1E), indicating *PRR11* as a predictor of prognosis.

This study evaluated whether *PRR11* expression levels were correlated with several clinical parameters in patients with lung adenocarcinoma. A total of 539 lung adenocarcinoma samples were obtained from TCGA, including *PRR11* expression data from various clinical conditions. The analysis revealed that *PRR11* expression levels were significantly correlated with pathological stage (III *vs.* I, $P < 0.001$), T grade (T2 *vs.* T1, $P < 0.001$), and N grade (N1 + N2 + N3 *vs.* N0, $P < 0.001$) (Figure 2A–C). Logistic regression analysis revealed that *PRR11* expression was associated with adverse clinicopathological and prognostic characteristics (Supplementary Table S3, available in www.besjournal.com). *PRR11* expression level was significantly correlated with the pathological stages, T grade and N grade ($P < 0.01$) in lung adenocarcinoma tissues. High *PRR11* expression was associated with more aggressive, poorly developed tumor morphologies and stage progression in patients.

GSEA showed significant differences for samples with high *PRR11* levels in GO and KEGG pathway enrichment (FDR < 0.05, P < 0.05). GO functional analysis revealed five categories positively associated with high *PRR11* expression (Figure 2D): mitotic sister chromatid segregation, sister chromatid separation, nuclear chromosome segregation, chromosome segregation, and condensed chromosomes. KEGG pathway analysis revealed five pathways positively associated with *PRR11* expression (Figure 2E): cell cycle, DNA replication, spliceosome, proteasome, and homologous recombination. These findings suggest that pathways regulating cell cycle control and amino acid metabolism are critical in the development of lung adenocarcinoma. *PRR11* mRNA expression was significantly higher in lung adenocarcinoma tissues than that in normal tissues ($|Log_2FC| > 1$, $P < 0.001$) (Figure 2F). As shown in Figure 2G, *PRR11* mRNA levels were correlated with poor overall survival ($P < 0.01$). *PRR11* expression was higher in lung adenocarcinoma tissues than that in non-neoplastic tissues by HPA immunohistochemistry (Figure 2H).

We investigated whether tissue *PRR11* expression correlated with immune infiltration using TIMER. As shown in Figure 3A, *PRR11* expression was positively correlated with CD8+T cells ($P = 5.58 \times 10^{-7}$), neutrophils ($P = 2.79 \times 10^{-12}$) and dendritic cells ($P = 2.95 \times 10^{-5}$), thus indicating *PRR11* as crucial for immune cell infiltration in LUAD. ssGSEA revealed that the levels of *PRR11* mRNA were

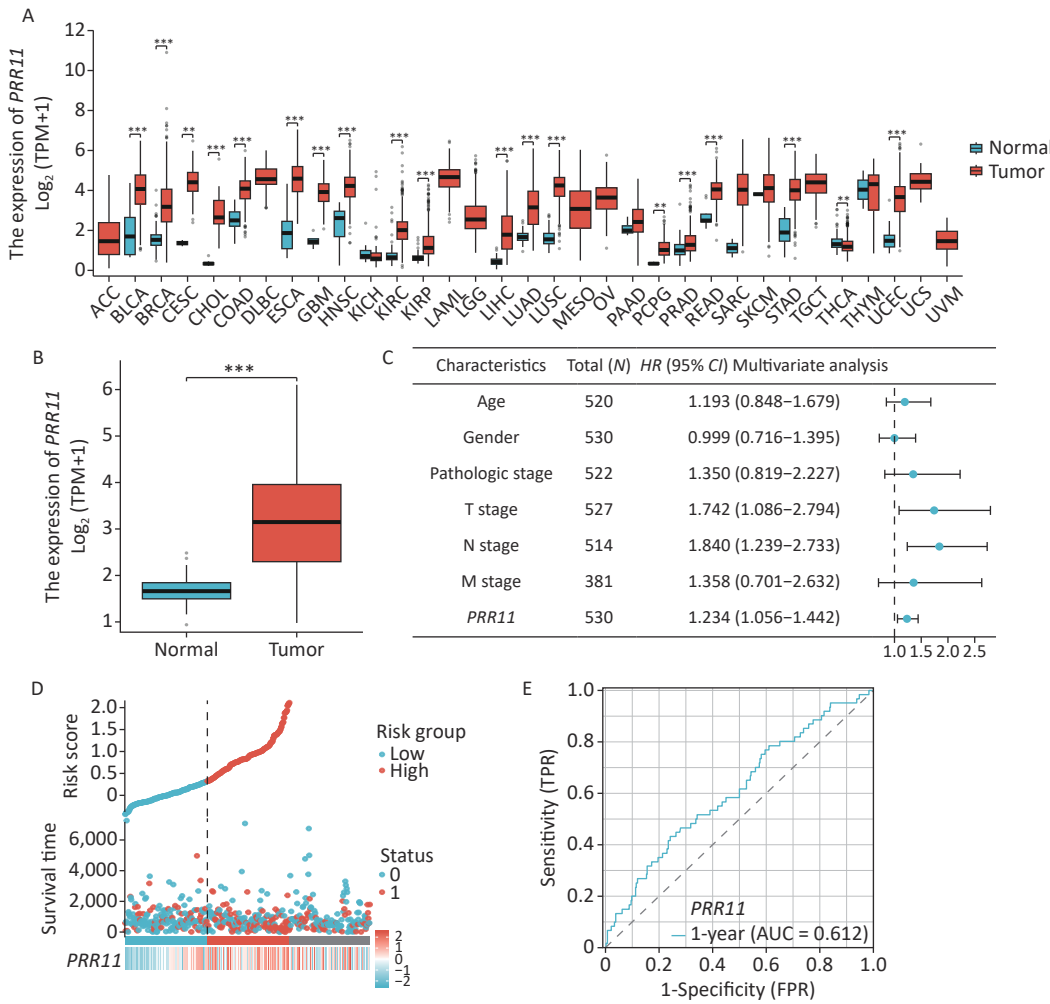

Figure 1. Expression and survival analysis of *PRR11* in lung adenocarcinoma. (A) The expression of *PRR11* in pan-cancer; (B) Differences in *PRR11* expression between normal and LUAD tissues; (C) *PRR11* expression and clinicopathological variables examined using Multivariate Cox regression; (D) Distribution of *PRR11* expression and survival status; (E) An analysis of *PRR11* in LUAD using ROC curve. (**, $P < 0.01$; ***, $P < 0.001$)

correlated with immune cell infiltration levels in LUAD. As shown in Figures 3B and 3C, a significant positive correlation was found between *PRR11* mRNA levels and immune cell abundance [T helper 2 (Th2) cells] ($P < 0.001$). In addition, our heat map assessing correlations among the 24 immune cell types (Figure 3D) revealed an important relationship between different subsets of tumor-infiltrating

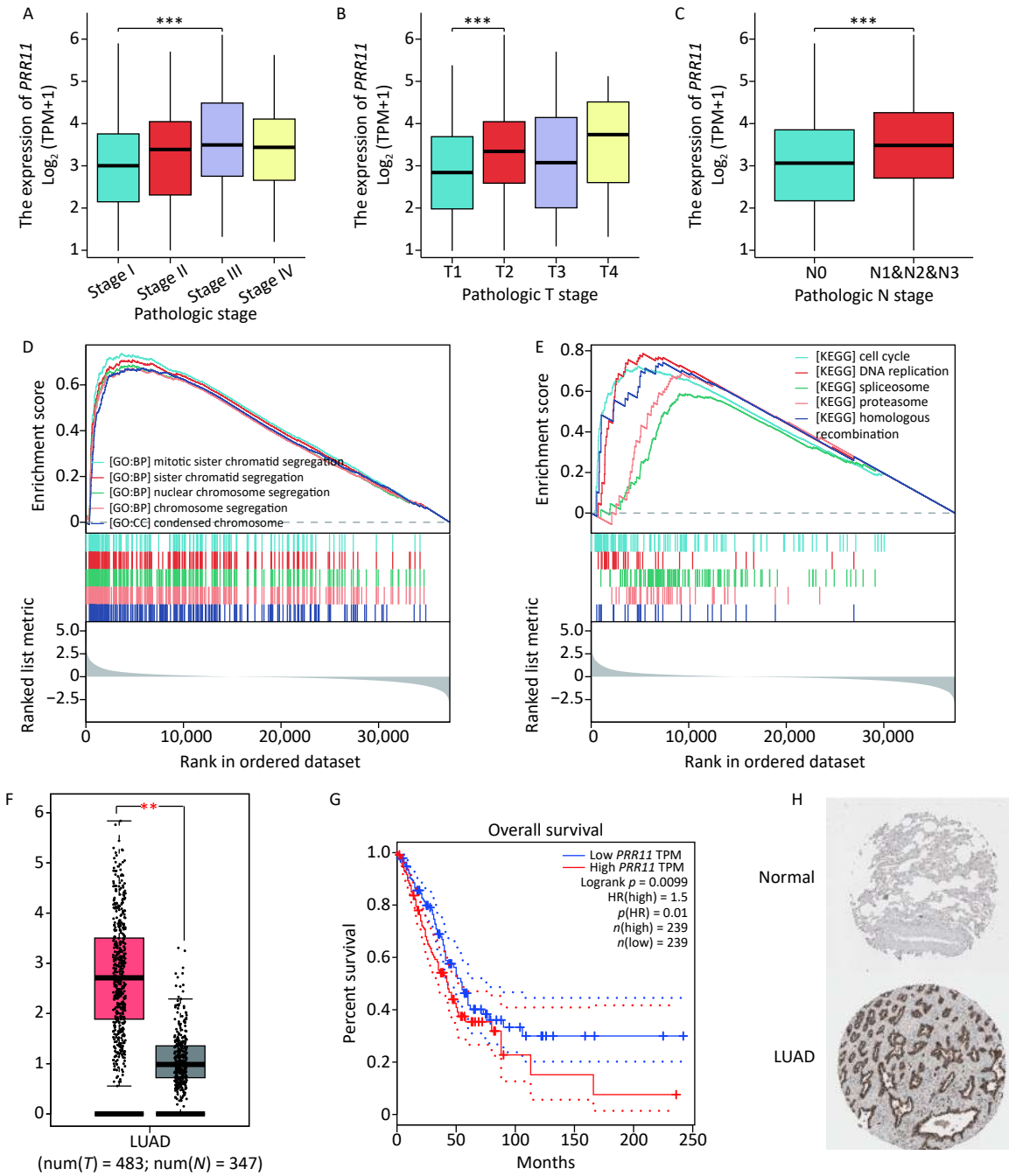

Figure 2. Analysis of *PRR11* expression and clinicopathological factors, *PRR11* enrichment analysis, and verification of data results. (A–C) *PRR11* expression showed a significant correlation with histological pathologic grade (A), T stage (B), and N stage (C); (D–E) GO and KEGG enrichment pathway analysis; (F–G) The comparison of *PRR11* expression levels and overall survival based on GEPIA data; (H) Immunohistochemical analysis of lung adenocarcinoma expression of the *PRR11* protein was conducted using HPA. (**, $P < 0.01$; ***, $P < 0.001$)

immune cells.

LUAD is one of the most aggressive cancers due to the associated mortality rate, which has gradually increased in prevalence. The development of

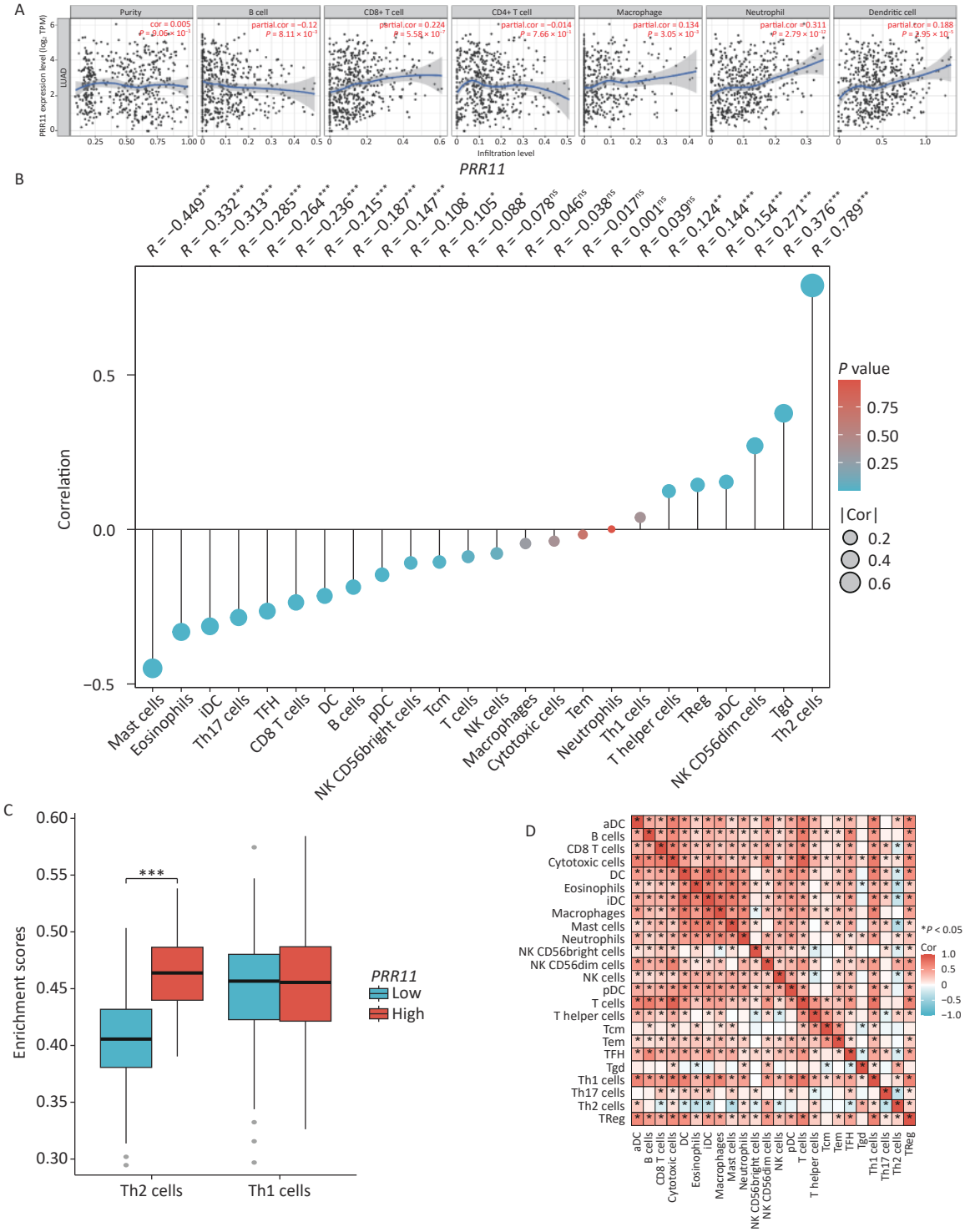

Figure 3. Association between *PRR11* and immune infiltration. (A) Relationship between *PRR11* and immune cells in TIMER; (B) Correlation between the relative abundances of 24 immune cell types and *PRR11* levels; (C) Differences between tumor samples with high and low *PRR11* expression in Th2 and Th1 cell proportions; (D) Heatmap of 24 immune infiltration cells in LUAD samples. (***, $P < 0.001$)

targeted therapy and immunotherapy has benefited many patients with LUAD by significantly prolonging survival[4]. The identification of meaningful gene targets for LUAD is valuable for guiding diagnosis and treatment[5]. Previous research has shown that *PRR11* expression is markedly low in normal tissues but significantly upregulated in some tumors[6]. In the current study, the cell cycle was significantly arrested and the ability of cells to invade and migrate was reduced after *PRR11* knockdown. Further, microarray analysis revealed the downregulation of key genes in several important pathways involved in cell cycle regulation, tumorigenesis, and metabolism. Notably, *PRR11* has been validated to be associated with tumor initiation and progression and can be used as a prognostic indicator for a variety of tumors[7].

Using bioinformatics tools and methods, data on lung adenocarcinoma subtypes were obtained from 59 normal and 539 tumor tissue samples and subsequently analyzed. Studies have investigated *PRR11* expression as a prognostic biomarker for lung adenocarcinoma. Based on TCGA data, *PRR11* was assessed for its prognostic value in patients with lung adenocarcinoma. The association of *PRR11* upregulation with various tumor characteristics and immune responses revealed its prognostic significance in patients with poor OS. In lung adenocarcinoma patients with high *PRR11* expression, stage and tumor status were more advanced than those in lung adenocarcinoma patients with low expression. Thus, high levels of *PRR11* expression may affect the progression of lung adenocarcinoma and the immune response.

PRR11 upregulation was predominantly related to the cell cycle and amino acid metabolism pathways based on GO function and KEGG pathway analysis. *PRR11* is known to be involved in signaling pathways related to cell cycle progression and may be closely associated with malignant tumor development in certain cancers, such as lung cancer. Silencing *PRR11* expression in lung cancer cells inhibited tumor growth and arrested the cell cycle. Consequently, cell cycle regulatory genes (e.g., CCNA1, CCNA2, and CDK6) were altered. The discovery of the biological processes and pathways in which *PRR11* is involved in regarding LUAD may help verify its role in promoting cancer progression.

According to the TIMER database, *PRR11* expression was associated with immune cell infiltration in lung adenocarcinoma. *PRR11* is strongly associated with CD8+T cells, neutrophils, and dendritic cells. Furthermore, our analysis revealed a significant relationship between *PRR11* expression and immune cell infiltration, particularly of Th2 cells, thus potentially revealing the mechanism by which *PRR11* regulates Th1/Th2 cell function in lung cancer. Helper T cells (Th cells) are important immunoregulatory cells that can be categorized into two main types: Th1 and Th2[8]. Th1 can produce cytokines such as IL-2, IFN-γ, TNF-β and others, which participate in cellular immunity. Th2 cells can produce cytokines such as IL-4, IL-5, and IL-10, which mediate humoral immunity. In particular, Th1 cells inhibit tumor growth by producing proinflammatory cytokines such as IFN-γ and IL-2, whereas Th2 cells produce anti-inflammatory cytokines such as IL-4 and IL-10 that promote tumor growth and metastasis[9]. Cellular immunity is a major component of the human immune response against cancer[10]. However, tumors have an immune escape mechanism that can lead to immune dysfunction, which allow them to evade or avoid immune strikes. In the lung cancer microenvironment, a shift in the Th1/Th2 balance is closely associated with tumor progression. Lung cancer cells can interact with Th1 and Th2 cells and cause a shift in Th1/Th2 balance, consequently accelerating lung cancer metastasis and progression. In this study, *PRR11* and Th2 cells decreased, thus indicating the superiority of humoral immunity, inhibiting the secretion of cytokines and cellular immune function of the body, and enabling tumor cells to survive and proliferate. The Th1/Th2 balance shift makes tumor cells more vulnerable to immune surveillance and attack, and accelerates the metastatic progression of lung adenocarcinoma. These findings suggest that *PRR11* is important for the regulation and recruitment of immune-infiltrating cells in lung cancer. However, to better understand the relationship between *PRR11* and Th1/Th2 cell balance in vivo, controlled experiments and multicenter clinical trials are necessary.

In conclusion, this study used bioinformatic techniques to identify *PRR11* as a biomarker in lung adenocarcinoma. As a regulatory and immune cell-infiltrating factor, *PRR11* appears to be associated with a poor prognosis in LUAD. With further investigation into its functional scope, *PRR11* may be a potential target for the diagnosis and treatment of lung adenocarcinomas.

Conflicts of Interest The authors have no conflicts of interest to declare.

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