Original Article

High expression of glycolysis-related *PGM2* gene in relation to poor prognosis and deficient immune cells infiltration in lung adenocarcinoma: a study based on bioinformatics analysis

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Background: Lung adenocarcinoma (LUAD) is the most important subtype of lung cancer and usually metastasizes. Patients with LUAD usually had a poor prognosis. Identifying viable molecular markers for diagnostic and prognostic prediction among individuals with LUAD is critical for the future management of this disease. This study aimed to determine and verify a correlation between the glycolysis-related phosphoglucomutase 2 (*PGM2*) gene and dissatisfactory results and deficient infiltration of immune cells in LUAD.

Methods: The expression of *PGM2* in LUAD and adjoining normal tissues was screened from The Cancer Genome Atlas (TCGA) data and human protein atlas (HPA), and validatied by quantative reverse transcription polymerase chain reaction (qRT-PCR). We examined the correlation between *PGM2* expression and clinicopathologic characteristics (including pathological stage, gender, M stage, smoker, age, N stage, race, and number pack years smoked) by multivariable approaches and Kaplan-Meier survival curves. The proteins network with *PGM2* was built using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database. The correlation between *PGM2* expression and infiltration of immune cells, along with the corresponding gene marker sets, was investigated through the Gene Expression Profiling Interactive Analysis (GEPIA) and Tumor Immune Estimation Resource (TIMER) databases. We evaluated the possible correlation between *PGM2* expression and progression-free interval (PFI), disease-specific survival (DSS), and overall survival (OS) in LUAD patients.

Results: Expression of *PGM2* was up-regulated in LUAD tissues (P=0.003). According to the multivariate logistic regression analysis, the elevated expression level of PGM2 exhibited a remarkable correlation with advanced tumor-node-metastasis (TNM) stage, high-grade malignancy, and primary therapeutic outcome . Overexpression of *PGM2* was shown to be correlated with an unfavorable prognosis including OS (P=0.004, HR =1.54), DSS (P=0.003, HR =1.77), and PFI (P=0.003, HR =1.5) in LUAD. The proteins PGM1 and UGP2 were shown to have a significant correlation with *PGM2*. Additionally, *PGM2* was associated with the lack of infiltrating immune cells as well as their associated gene marker sets in LUAD.

Conclusions: Overexpression of *PGM2* was shown to be associated with the progression and an unfavorable prognosis of LUAD, as well as with inefficient immune cell infiltration. *PGM2* was expected to be a potential biological marker for predicting the prognosis of patients with LUAD.

Keywords: Phosphoglucomutase 2 (PGM2); lung adenocarcinoma (LUAD); glycolysis; immune cell infiltration

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Introduction

Based on the latest international cancer figures, lung cancer is the utmost universally diagnosed tumor (11.6% of the overall cases) and the major contributor to cancer-related fatalities (18.4% of the overall malignancy deaths) (1). Primary lung malignancy is typically classified into non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). Around 83% of all lung cancer cases are NSCLC and lung adenocarcinoma (LUAD) is the most prevalent subtype of NSCLC. Virtually 80% of NSCLC patients are diagnosed at an advanced phase due to the lack of initial detection biomarkers (2-5), and those with advanced lung cancer have a poor prognosis. LUAD exhibits relatively poor prognosis warranting the need for better predictors of clinical outcome and treatment strategies. Consequently, there is an urgent need to distinguish potential biological markers to improve prognosis of LUAD. Multiple factors participate in the development of LUAD including histological, metabolic, and genetic mechanisms; one of the chief features is energetical glucose metabolism. Compared with normally differentiated cells, the metabolism of cancer cells relies on glycolysis instead of mitochondrial oxidative phosphorylation (OXPHOS), consumes glucose at a higher degree, and yields more lactic acid to meet their higher requirement for nutrients (6-8). The alteration of energy metabolism, referred to as aerobic glycolysis or the Warburg effect, is considered a typical hallmark of malignancy (9-11). The Warburg effect is beneficial to tumor progression, proliferation, and metastasis and plays a crucial role in tumor vitality, hence aerobic glycolysis is a critical energy generation pathway for cancer cells, where the molecules involved may convert the potential biomarkers and therapeutic targets for LUAD (12).

Dysregulated expression of glycolysis-related genes (GRGs) is strongly correlated with the progression of various malignancies, and it is being considered as a possible target for the treatment of tumors. Cui *et al.* acquired 226 differentially expressed central GRG (DE-GRGs) from The Cancer Genome Atlas (TCGA) database, 8 of which were nominated as central DE-GRG using Cox regression analysis to develop a DE-GRG prognostic model, of which risk score is closely associated with multiple clinical features and is an independent hazard factor affecting prognosis. Phosphoglucomutase 2 (*PGM2*), is one of the central DE-GRG (13). Zhang *et al.* similarly uncovered that *PGM2* expresses abnormally in clear renal cell carcinoma and performs a crucial assessment function as a GRG for the diagnosis and prognostic evaluation of clear renal cell carcinoma (14).

In the present study, we focused on the function of the GRG *PGM2* in LUAD. We evaluated the expression of *PGM2* in LUAD tissues and its correlation with LUAD stage, pathologic grade, and prognosis. The function of *PGM2* in the infiltration of immune cells in LUAD was also reported in this study. It is anticipated that *PGM2* may serve as a prognostic biomarker for LUAD. We present the following article in accordance with the REMARK reporting checklist (available at https://jtd.amegroups.com/article/view/10.21037/jtd-22-1043/rc).

Methods

TCGA database analysis

The information of LUAD patients comprising both RNA sequencing data and clinicopathological factors (including pathological stage, gender, TNM stage, smoking status, age, race, and number pack years smoked) was acquired from the TCGA (https://genomecancer.ucsc.edu/) database, which is a widely used data platform for large-scale cancer genome projects.

Oncomine database analysis and the Human Protein Atlas

The Oncomine database (https://www.oncomine.org/resource/login.html) is the most comprehensive cancer-associated gene microarray platform and cohesive data-resource portal in the world. Oncomine allows users to search for the fullest spectrum of cancer mutations, respective clinical data, and gene expression profiles. We made a comparison of the *PGM2* gene expression levels in cancer and normal tissues. By merging several omics methods, the Human Protein Atlas (HPA; https://www.proteinatlas.org/) provides data on all human protein transcripts from unique human samples encompassing cells, tissue, and organs. We obtained *PGM2* protein immunohistochemistry in LUAD (https://www.proteinatlas.org/ENSG00000169299-PGM2/pathology/lung+cancer) and normal human samples (https://www.proteinatlas.org/ENSG00000169299-PGM2/tissue/lung) from the HPA (images are available from v21.1.proteinatlas.org). When the fold change (FC) was >1.5 and the P value <0.001, the expression levels were considered substantially different in various tissues. The gene rank criterion was determined to be "top 10%", where the data type was set to "all".

The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database

The STRING platform (https://string-db.org/) has a large amount of data on protein-protein interactions (PPIs). To investigate *PGM2*-related proteins, we created a PPI network of *PGM2* with the aid of the STRING database. The significant criterion was established as a confidence score greater than 0.7.

Survival and statistical analysis

We classified patients into low- and high-*PGM2* expression groups based on their median *PGM2* gene expression levels. Relevant gene expression profiles and relevant clinical information was collected by Xiantao platform (www.xiantao.love).With the use of the Kaplan-Meier plotter database (https://kmplot.com/analysis/), we evaluated the possible correlation between *PGM2* expression and progression-free interval (PFI), disease-specific survival (DSS), and overall survival (OS) in LUAD patients. The following were the selection thresholds: log-rank P value <0.05 (two-sided) and hazard ratio (HR) within the 95% confidence interval (CI).

Correlation analysis regarding immune cell infiltration

The Tumor Immune Estimation Resource (TIMER) (cistrome.org/TIMER/), a publicly accessible web platform containing data on 32 kinds of cancer and incorporating 10,897 samples from the TCGA database, was utilized to assess the correlation between *PGM2* expression and the abundance of 6 infiltrating immune cells (neutrophils, $CD4^+$ T cells, dendritic cells, B cells, macrophages, and $CD8^+$ T cells) among patients with LUAD. We also observed the correlation between *PGM2* gene expression and the marker genes of tumor-infiltrating immune cells (TIICs) via the TIMER website.

Quantitative real-time polymerase chain reaction

A total of 12 pairs of clinical LUAD samples and adjacent lung tissues of primary LUAD patients from Wuxi People's Hospital were collected during surgery and instantly frozen in liquid nitrogen and preserved at −80 ℃ for further use. Total RNA from tissues was extracted using TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA). The RNA was then reverse-transcribed into complementary DNA (cDNA) according to the manufacturer's instructions. Then, we performed quantitative real-time polymerase chain reaction (qRT-PCR) using SYBR Green Real-time PCR Master Mix (Thermo Fisher, USA). Relative gene expression was calculated using the 2^{-DDCq} method. The primer sequences were displayed as follows: *PGM2*, F: GAGGCAGTGAAACGACTAATAGC, R: CTGTCCCAAACTCCATTCGGG; GAPDH, F: CCTTCCGTGTCCCCACT, R: GCCTGCTTCACCACCTTC. This study was approved by the Institutional Research Ethics Committees of Wuxi People's Hospital Affiliated to Nanjing Medical University (No. KY21075), and informed consent was taken from all the patients. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Statistical analysis

The software SPSS (IBM Corp., Armonk, NY, USA) and GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA) were utilized to perform analyses of all statistical data. All data were expressed as means ± standard deviations. For the purpose of determining the significance level of difference across the 2 groups, 2-tailed Student's *t*-tests were carried out. Results with P values <0.05 (two-sided) were interpreted as statistically significant unless otherwise specified.

Results

Baseline characteristics of patients

In total, 535 LUAD and 59 normal tissue samples were included in the present study from the TCGA database. Among the 535 participants, 286 patients were female (53.5%) and 249 patients were male (46.5%). Some 446 patients were smokers (85.6%). In regards to the pathological stage, 294 partcipants (55.8%) were already in stage I, 123 patients (23.3%) were in stage II, 84 patients (15.9%) were in stage III, and 26 patients were in stage IV (4.9%). The detailed clinical information is listed in *Table 1*.

PGM2 expression level in LUAD and other malignancies

To investigate the involvement of *PGM2* in oncogenesis, we performed Oncomine analysis to explore the *PGM2* messenger RNA (mRNA) level in different kinds of cancer. The results illustrated that the *PGM2* gene was down-

Table 1 Clinical features of patients with lung adenocarcinoma

Characteristics	Low expression of *PGM2*	High expression of *PGM2*	Overall
n	267	268	535
T stage, n (%)			
T1	105 (19.7)	70 (13.2)	175 (32.9)
T2	127 (23.9)	162 (30.5)	289 (54.3)
T3	25 (4.7)	24 (4.5)	49 (9.2)
T4	9 (1.7)	10 (1.9)	19 (3.6)
N stage, n (%)			
N0	192 (37)	156 (30.1)	348 (67.1)
N1	39 (7.5)	56 (10.8)	95 (18.3)
N2	27 (5.2)	47 (9.1)	74 (14.3)
N3	1 (0.2)	1 (0.2)	2 (0.4)
M stage, n (%)			
M0	178 (46.1)	183 (47.4)	361 (93.5)
M1	9 (2.3)	16 (4.1)	25 (6.5)
Pathologic stage, n (%)			
Stage I	165 (31.3)	129 (24.5)	294 (55.8)
Stage II	54 (10.2)	69 (13.1)	123 (23.3)
Stage III	35 (6.6)	49 (9.3)	84 (15.9)
Stage IV	9 (1.7)	17 (3.2)	26 (4.9)
Primary therapy outcome, n (%)			
PD	25 (5.6)	46 (10.3)	71 (15.9)
SD	21 (4.7)	16 (3.6)	37 (8.3)
PR	2 (0.4)	4 (0.9)	6 (1.3)
CR	181 (40.6)	151 (33.9)	332 (74.4)
Gender, n (%)			
Female	152 (28.4)	134 (25)	286 (53.5)
Male	115 (21.5)	134 (25)	249 (46.5)
Race, n (%)			
Asian	4 (0.9)	3 (0.6)	7 (1.5)
Black or African American	36 (7.7)	19 (4.1)	55 (11.8)
White	202 (43.2)	204 (43.6)	406 (86.8)
Age, n (%)			
≤65	129 (25)	126 (24.4)	255 (49.4)
>65	131 (25.4)	130 (25.2)	261 (50.6)

Table 1 *(continued)*

Table 1 *(continued)*

Characteristics	Low expression of *PGM2*	High expression of *PGM2*	Overall
No. of pack years smoked, n (%)			
<40	99 (26.8)	89 (24.1)	188 (50.9)
≥40	86 (23.3)	95 (25.7)	181 (49.1)
Smoker, n (%)			
No	38 (7.3)	37 (7.1)	75 (14.4)
Yes	225 (43.2)	221 (42.4)	446 (85.6)

PGM2, phosphoglucomutase 2.

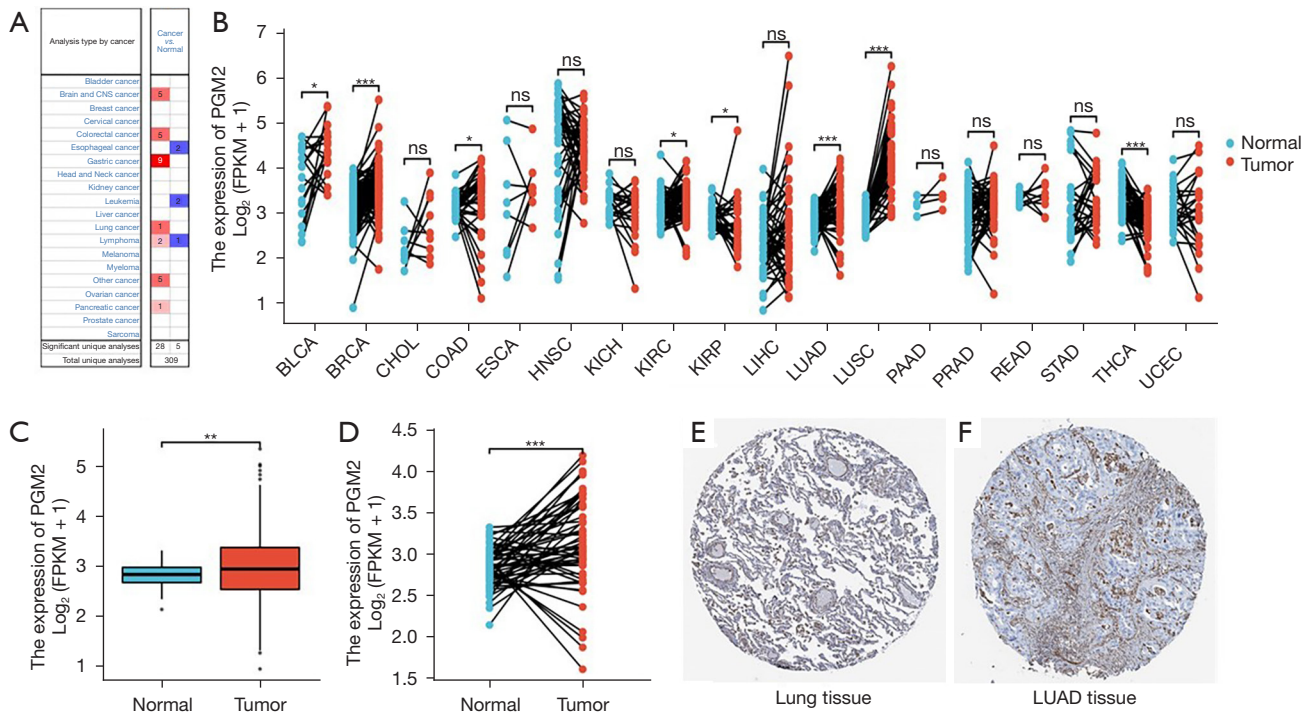


Figure 1 Expression analysis of *PGM2* in LUAD and other cancer types. (A) *PGM2* expression in distinct types of human cancers in the Oncomine database; (B) *PGM2* expression in distinct kinds of human cancers in TCGA database; (C) *PGM2* expression in unpaired samples of LUAD and adjoining normal lung samples in TCGA database; (D) *PGM2* expression in paired samples of LUAD and adjoining normal lung samples in the TCGA database; The *PGM2* protein expression levels by immunohistochemistry in LUAD sample (E) was elevated as opposed to those in normal samples (F) in the HPA (antibody HPA040676, 10×). *Figure 1E* (https://www.proteinatlas.org/ENSG00000169299-PGM2/tissue/lung) and *Figure 1F* (https://www.proteinatlas.org/ENSG00000169299-PGM2/pathology/lung+cancer) are from the HPA (images are available from v21.1.proteinatlas.org). ns, P≥0.05; *P<0.05; **P<0.01; ***P<0.001. *PGM2*, phosphoglucomutase 2; FPKM, fragments per kilobase of exon model per million mapped fragments; LUAD, lung adenocarcinoma; TCGA, The Cancer Genome Atlas; HPA, Human Protein Atlas.

regulated in esophageal cancer and leukemia. However, *PGM2* gene expression was found to be remarkably upmodulated in lung cancer, central nervous system cancer, pancreatic cancer, brain cancer, gastric cancer, and colorectal cancer. Subsequently, the expression level of *PGM2* mRNA in lymphoma was elevated in 2 studies and decreased in 1 study (*Figure 1A*). Meanwhile, *PGM2* was expressed at a high level in 5 kinds of cancers, such as lung

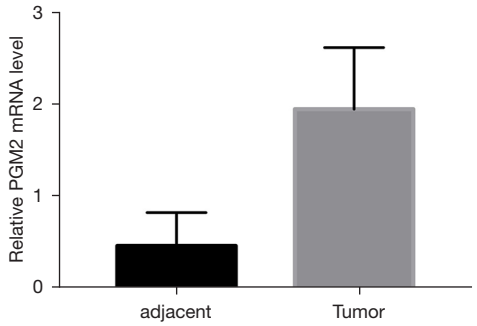


Figure 2 Relative *PGM2* mRNA expression level. qRT-PCR result showed that *PGM2* mRNA expression in LUAD primary tumor was higher than that in adjacent normal tissues (P<0.01, n=12). *PGM2*, phosphoglucomutase 2; qRT-PCR, quantitative real-time polymerase chain reaction; mRNA, messenger RNA; LUAD, lung adenocarcinoma.

squamous cell carcinoma (LUSC) and LUAD based on RNA sequence data found in the TCGA (*Figure 1B*). The mRNA expression level of *PGM2* was significantly elevated in tumor samples as opposed to unpaired normal LUAD samples in TCGA (*Figure 1C*, P=0.003). The results were verified in LUAD samples and paired normal lung samples (*Figure 1D*, P=0.001). Moreover, the expression of *PGM2* protein was up-regulated in LUAD samples in contrast with normal samples in the HPA (*Figure 1E,1F*). Besides, we performed qRT-PCR to validate the *PGM2* mRNA expression in LUAD and found that *PGM2* mRNA was up-regulated in the LUAD tumors (n=12) compared with normal lung tissues (n=12; P<0.001; *Figure 2*). Overall, these results strongly suggested that *PGM2* was up-regulated in LUAD and might perform a negative role in LUAD.

Association of PGM2 expression with clinical features

We assessed the correlation of *PGM2* expression with clinical features among patients with LUAD. Elevated expression level of *PGM2* was remarkably correlated with T stage (T2 *vs.* T1, $P_{.adj}$=0.002) and primary therapeutic outcome (CR *vs.* PD, $P_{.adj}$=0.042). There was no remarkable correlation detected between elevated expression level of of *PGM2* and other clinical features, including pathological stage, gender, M stage, smoker, age, N stage, race, and number pack years smoked (*Figure 3*). In univariate logistic regression analysis, elevated expression level of of *PGM2* exhibited a substantial correlation with T stage [T2, T3, and T4 *vs.* T1, odds ratio (OR) =1.826, 95% confidence interval (CI): 1.267–2.644, P=0.001], N stage (N1, N2, and N3 *vs.* N0, OR =1.910, 95% CI: 1.319–2.782, P<0.001), pathological stage (stage III and stage IV *vs.* stage I and stage II, OR =1.659, 95% CI: 1.086–2.555, P=0.020), and primary therapeutic outcome [stable disease (SD), partial response (PR), and complete response (CR) *vs.* progressive disease (PD); OR =0.456, 95% CI: 0.266–0.766, P=0.003), as shown in *Table 2*.

Association of enhanced PGM2 expression and unfavorable prognosis in patients with LUAD

We assessed the prognostic significance of *PGM2* in LUAD through Kaplan-Meier analysis. We found that enhanced *PGM2* expression was linked to unfavorable patient prognosis including OS (*Figure 4A*, P=0.004, HR =1.54, 95% CI: 1.15–2.06), DSS (*Figure 4B*, P=0.003, HR =1.77, 95% CI: 1.22–2.57), and PFI (*Figure 4C*, P=0.003, HR =1.50, 95% CI: 1.15–1.96) in LUAD. The Kaplan-Meier plotter in LUAD was used to analyze patients belonging to different groups by different clinical variables. The results demonstrated that enhanced *PGM2* expression was remarkably linked to shortened OS duration in LUAD cases in the T1/T2 stage (*Figure 4D*, P=0.006, HR =1.57, 95% CI: 1.14–2.18), N0/N1 satge (*Figure 4E*, P=0.037, HR =1.42, 95% CI: 1.02–1.98), male (*Figure 4F*, P=0.021, HR =1.64, 95% CI: 1.08–2.49), white race (*Figure* 4G, P=0.027, HR =1.45, 95% CI: 1.04–2.00), age over 65 years (*Figure 4H*, P=0.037, HR =1.53, 95% CI: 1.03–2.28), smokers (*Figure 4I*, P=0.008, HR =1.55, 95% CI: 1.12–2.14), and with no residual tumor (*Figure 4J*, P=0.009, HR =1.58, 95% CI: 1.12–2.22). The above findings illustrated the correlation between enhanced *PGM2* expression and unfavorable prognosis among patients with LUAD.

Constructing PPIs

We further sought PPIs to clarify the molecular mechanisms and metabolism of malignant tumors. The PPI network of PGM2 protein was assessed with the help of the STRING tool so as to evaluate their functional interplays in the progression of LUAD. These genes, along with their annotations and scores, are summarized in *Figure 5*. The 10 most common proteins, including *UGP2*, *GPI*, *RBKS*, *DERA*, *PNP*, *TKT*, *RPIA*, *PRPS2*, *HK1*, and *PGM1*, participate in both the breakdown and synthesis of glucose. In cellular metabolic pathways, *UGP2* serves as a glucose donor.

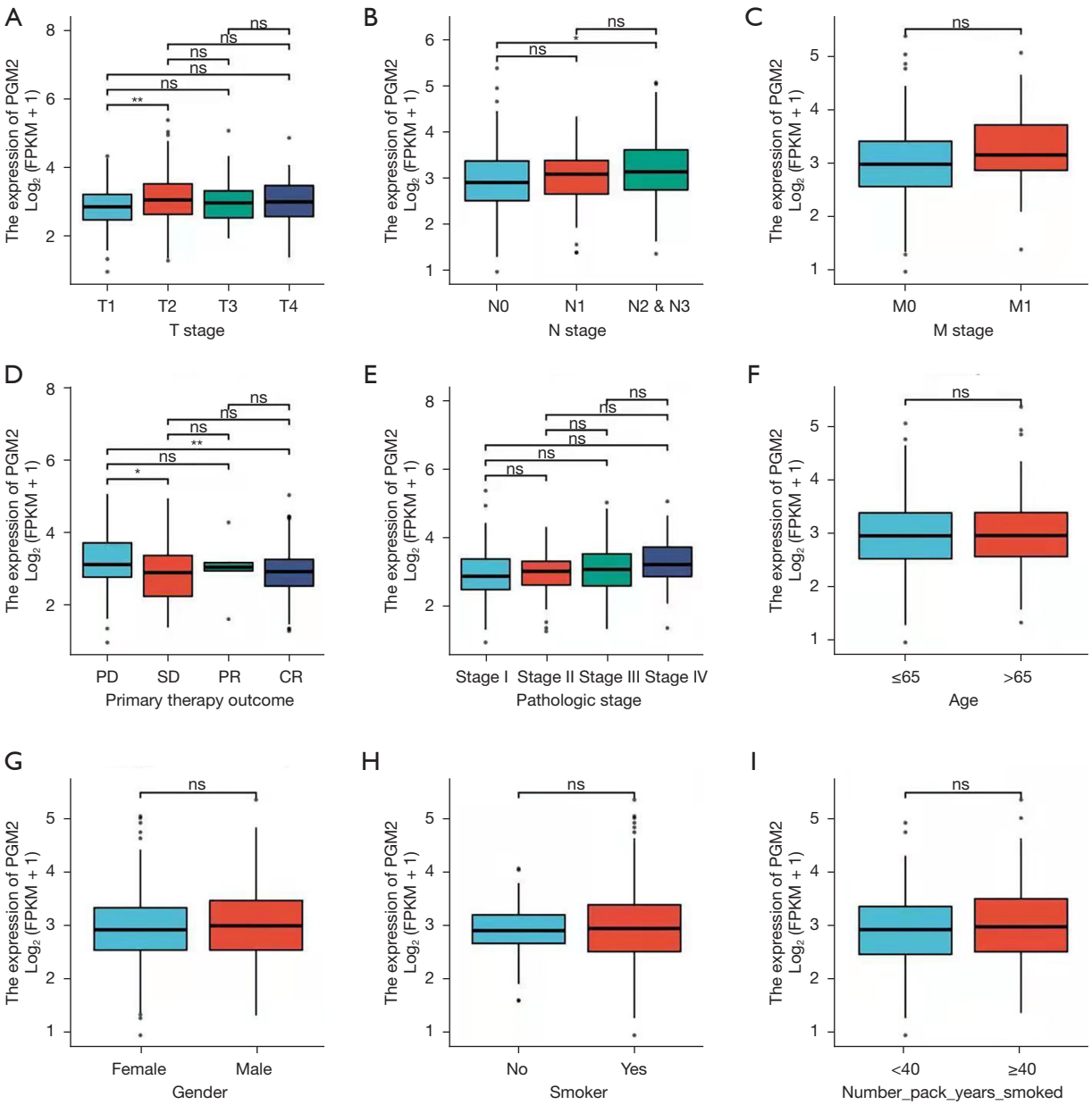


Figure 3 Box plot evaluating *PGM2* expression of patients with LUAD according to different clinical characteristics. (A) T classification ; (B) N classification; (C) M classification; (D) primary therapy outcome; (E) pathologic stage; (F) Age; (G) Gender; (H) Smoker; (I) number_pack_years_smoked. ns, P≥0.05; *P<0.05; **P<0.01; ***P<0.001. *PGM2*, phosphoglucomutase 2; LUAD, lung adenocarcinoma.

Assessment of the link between PGM2 expression and immune cells infiltration

Tumor-infiltrating lymphocytes (TILs) have been proposed as a prognostic marker in patients with multiple malignancies. In view of this, the correlation between *PGM2* expression of 6 kinds of infiltrating immune cells (neutrophils, macrophages, $CD8^+$ T cells, B cells, dendritic cells, and $CD4^+$ T cells) and tumor purity was analyzed. The findings illustrated a strong correlation between the *PGM2* expression and tumor purity (r=–0.096, P=3.29e-02), infiltration levels of B cells (r=–0.154, P=6.71e-04), dendritic cells (r=0.286, P=1.28e-10), $CD8^+$ T cells (r=0.289, P=8.07e-11), neutrophils (r=0.376, P=1.09e-17),

Table 2 Logistic analysis of the association between *PGM2* expression and clinical features

Characteristics	Total (N)	Odds ratio (95% CI)	P value
T stage (T2 & T3 & T4 *vs*. T1)	532	1.826 (1.267–2.644)	0.001
N stage (N1 & N2 & N3 *vs*. N0)	519	1.910 (1.319–2.782)	<0.001
M stage (M1 *vs*. M0)	386	1.729 (0.759–4.181)	0.203
Pathologic stage (stage III & stage IV *vs.* stage I & stage II)	527	1.659 (1.086–2.555)	0.020
Primary therapy outcome (SD, PR, CR *vs.* PD)	446	0.456 (0.266–0.766)	0.003
Gender (male *vs*. female)	535	1.322 (0.941–1.860)	0.108
Race (Black or African American & White *vs.* Asian)	468	1.249 (0.273–6.401)	0.772
Age (>65 *vs*. ≤65 years)	516	1.016 (0.719–1.435)	0.928
Residual tumor (R1 & R2 *vs.* R0)	372	2.671 (0.969–8.540)	0.070
Anatomic neoplasm subdivision (right *vs.* left)	520	1.009 (0.710–1.435)	0.959
Anatomic neoplasm subdivision 2 (peripheral lung *vs.* central lung)	189	1.359 (0.739–2.517)	0.325
Number of pack years smoked (≥40 *vs.* <40)	369	1.229 (0.817–1.851)	0.323
Smoker (yes *vs.* no)	521	1.009 (0.618–1.649)	0.972

PGM2, phosphoglucomutase 2; CI, confidence interval; SD, stable disease; PR, partial response; CR, complete response; PD, progressive disease.

macrophages (r=0.286, P=1.34e-10), and $CD4^+$ T cells (r=0.052, P=2.56e-01) in LUAD (*Figure 5*).

Correlation analysis of PGM2 mRNA expression levels with biomarkers of different immune cell subsets

In addition, we assessed the expression of biomarker genes in diverse immune cells or related subsets in LUAD tissues for the purpose of laying a foundation for future research into *PGM2*-driven carcinogenesis processes and the identification of viable treatment targets. The findings obtained from TIMER analysis revealed a negative correlation between *PGM2* expression and *CD19*, *CD79A*, *CEACAM8*, *HLA-DPB1*, *HLA-DQB1*, *CD1c*, and *CCR7*. Additionally, a positive correlation was observed between *ITGAX*, *VSIG4*, *CD163*, *MS4A4A*, *IL10*, *ITGAM*, *PTGS2*, *HLA-DRA*, *IRF5*, *HLA-DPA1*, *CD68*, *NRP1*, and *CCL2* (*Figure 6*, *Table 3*).

Discussion

Currently, NSCLC is the most common type of lung cancer which is branded by the dysregulation genetic material such as protein-coding genes and non-coding RNAs (4). Lung cancer is the primary cause of cancer-associated mortality and the survival of this cancer primarily relies upon the phase at diagnosis, though almost two-thirds of lung cancer patients are diagnosed at advanced stages. Our study revealed that the *PGM2* gene was remarkably up-regulated in LUAD, which was correlated with differential OS and poor prognosis. Our study similarly recommended that *PGM2* is associated with immune implications in LUAD, which may be the mechanism through which *PGM2* influences prognosis. The results of our research revealed that *PGM2* may become a prospective biomarker for diagnosis and prognosis which may serve an innovative immune-related therapeutic purpose.

The gene *PGM2* is one of the central DE-GRG (14). The results in our study suggested that *PGM2* was up-regulated in LUAD and might play a negative role in LUAD, which indicates that the expression of *PGM2* can distinguish LUAD patients from healthy individuals with extraordinary sensitivity and specificity. The elevated expression level of *PGM2* was remarkably linked to T stage, especially in T1, T2, and primary therapeutic outcome, but there was no remarkable correlation between the elevated expression level of *PGM2* and other clinical features, including M stage, pathologic stage, age, gender, smoking history, race, and number of pack-years smoked. These results indicate that *PGM2* has a vast diagnostic value for LUAD at the initial phase.

Kaplan-Meier analysis was performed for the purpose

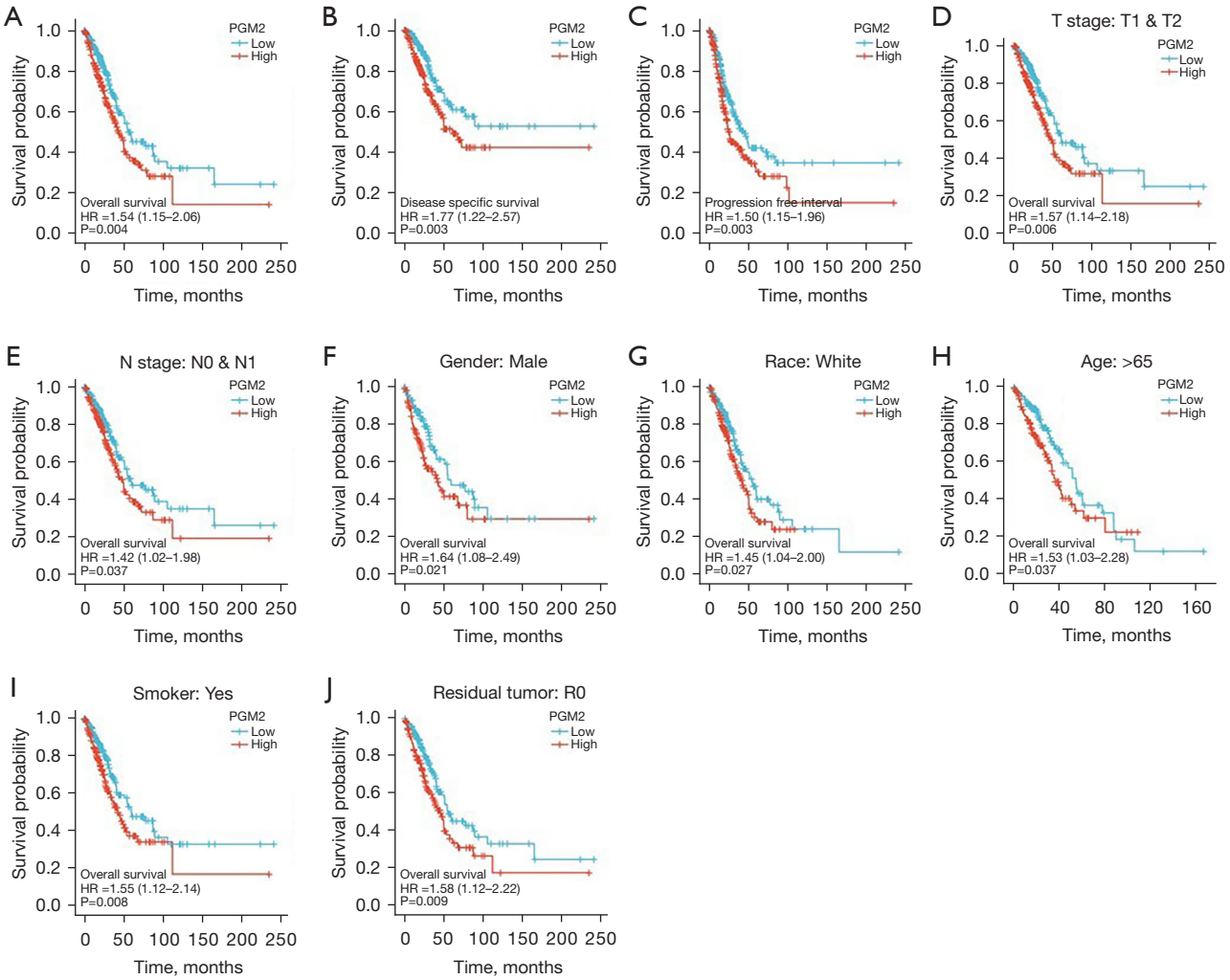
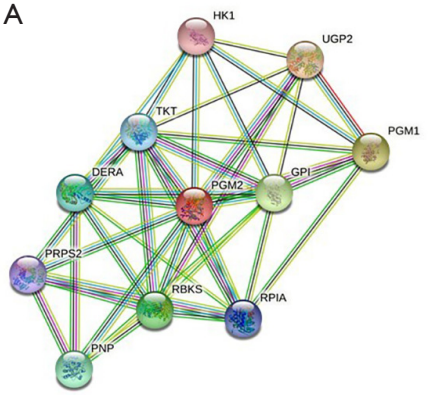


Figure 4 Correlation of *PGM2* expression and prognosis in LUAD patients with different clinical features via Kaplan-Meier plotter. (A) OS; (B) DSS; (C) PFI; (D-J) Subgroup analysis for OS for T1/T2 stage, N0/N1 stage, male, white, age over 65 years, smoker, and with no residual tumor. *PGM2*, phosphoglucomutase 2; LUAD, lung adenocarcinoma; OS, overall survival; DSS, disease-specific survival; PFI, progression-free interval.

of assessing *PGM2*'s prognostic significance in LUAD. Moreover, we discovered that enhanced *PGM2* expression was linked to an unfavorable prognosis in patients with LUAD, as validated by the values of DSS, OS, and PFI. The findings from subgroup analysis illustrated that enhanced expression of *PGM2* was substantially related to an unfavorable OS in LUAD cases in the T1/T2 stage, N0/N1 satge, and so on. Multivariate and univariate analyses revealed that enhanced expression of *PGM2* may be described as a hazard element that influences the OS of patients with LUAD. Thus, we confirmed that *PGM2* has prognostic value in LUAD.

Recently, with the upsurge of immunotherapy, the growth of immune-checkpoint inhibitors (ICIs) has intensely transformed the NSCLC medicinal domain. The progress of immunotherapeutic strategies transforms the stability of immune viability away from a cancer-induced immune oppressive state to a vigorous antitumor immunity response (15,16). The findings from the present study illustrated a correlation between the expression of *PGM2* and tumor purity, as well as infiltrating levels of macrophages, $CD4^+$ T cells, dendritic cells, B cells, $CD8^+$ T cells, and neutrophils in LUAD. By performing TIMER analysis, we discovered a negative correlation between *PGM2* expression and *CD19*,

A



B

Gene symbol	Annotation	Score
PGM1	Phosphogucomutase-1	0.98
UGP2	UTP-ghucose-1-phosphate uridylyltransferase	0.98
GPI	Glucose-6-phosphate isomerase	0.97
RBKS	Ribokinase	0.962
DERA	Deoxyribose-phosphate aldolase	0.959
PNP	Purine nucleoside phosphorylase	0.959
TKT	Transketolase	0.955
RPIA	Ribose 5-phosphate isomerase A	0.953
PRPS2	Ribose-phosphate pyrophosphokinase 2	0.935
HK1	Catalyzes the phosphorylation of various hexoses	0.933

Figure 5 Constructing protein interaction networks of *PGM2*. (A) *PGM2*-interaction proteins in LUAD tissue. (B) Annotation of *PGM2*-interacting proteins and their co-expression scores. *PGM2*, phosphoglucomutase 2; LUAD, lung adenocarcinoma.

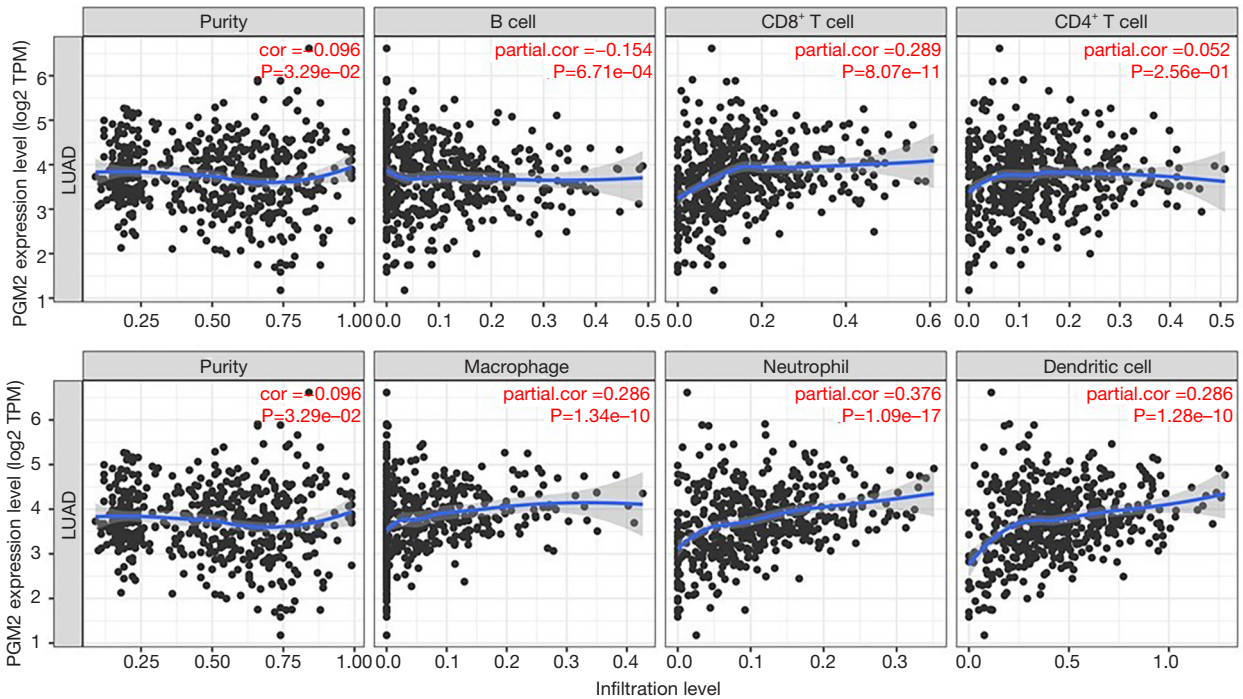


Figure 6 Correlation analysis between *PGM2* expression and immune cell infiltration levels in LUAD via TIMER analysis (n=371). *PGM2*, phosphoglucomutase 2; LUAD, lung adenocarcinoma; TIMER, Tumor Immune Estimation Resource.

HLA-DQB1, *CEACAM8*, *CD79A*, *HLA-DPB1*, *CD1c*, and *CCR7*. Additionally, a positive correlation was observed between *ITGAX*, *IL10*, *HLA-DPA1*, *PTGS2*, *ITGAM*, *VSIG4*, *CD163*, *MS4A4A*, *IRF5*, *HLA-DRA*, *CD68*, *NRP1*, and *CCL2*. The T lymphocytes are the most common immune cell types in the LUAD cancer microenvironment, followed by B cells, natural killer cells, and macrophages (17). Dendritic cells are individual antigen-presenting cells that perform a specific function to the incentive of anticancer T lymphocytes (18,19). The influence of T cells universally predicts a superior theoretical consequence in LUAD (20). Additionally, the function of cancer-infiltrating B cells in the microenvironment of cancer has triggered growing responsiveness. The majority of studies point out that the influence of B cells in LUAD is linked to a satisfactory consequence (21,22).

Table 3 Correlation analysis between *PGM2* and related genes and markers of immune cells via TIMER

Description	Gene markers	Purity	
		Correlation	P value
B cell	CD19	–0.168	1.75e–04*
	CD79A	–0.143	1.45e–03*
Tumor-associated macrophage	CCL2	0.24	6.90e–08*
	CD68	0.363	8.88e–17*
	IL10	0.271	9.69e–10*
M1 Macrophage	IRF5	0.248	2.46e–08*
	PTGS2	0.152	6.81e–04*
M2 Macrophage	CD163	0.361	1.16e–16*
	VSIG4	0.275	4.94e–10*
	MS4A4A	0.28	3.29e–02*
Neutrophils	CEACAM8	–0.064	1.55e–01*
	ITGAM	0.25	1.86e–08*
	CCR7	–0.033	4.61e–01*
Dendritic cell	HLA-DPB1	–0.016	7.19e–01*
	HLA-DQB1	–0.085	5.98e–02*
	HLA-DRA	0.07	1.22e–07*
	HLA-DPA1	0.048	2.91e–01*
	CD1c	–0.078	8.36e–02*
	NRP1	0.177	7.57e–05*
	ITGAX	0.125	5.30e–03*
$CD4^+$ T cells			

*P<0.05. *PGM2*, phosphoglucomutase 2; TIMER, Tumor Immune Estimation Resource.

In conclusion, we discovered that *PGM2* is upregulated in LUAD tissues and that elevated expression level of *PGM2* is correlated with clinical progression and can serve as a risk factor for OS in LUAD patients in an independent manner. Immunotherapy has been the most significant advancement in LUAD treatment. The findings of the present study illustrated that a higher expression level of the glycolysis-related *PGM2* gene was associated with lower immune cell infiltration. Moreover, *PGM2* might influence glycolysis pathways in LUAD, which might be crucial in cancer. Additionally, *PGM2* might serve as a marker for helping to identify patients who may be candidates for glycolysis-induction therapy or its integration with immunotherapy. Taken together, *PGM2* is a potential biological marker for predicting the prognosis of patients with LUAD.

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Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://jtd.amegroups.com/article/view/10.21037/jtd-22-1043/rc

Data Sharing Statement: Available at https://jtd.amegroups.com/article/view/10.21037/jtd-22-1043/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jtd.amegroups.com/article/view/10.21037/jtd-22-1043/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Institutional Research Ethics Committees of Wuxi People's Hospital Affiliated to Nanjing Medical University (No. KY21075), and informed consent was taken from all the patients.

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