Original Article

LDHA as a predictive biomarker and its association with the infiltration of immune cells in pancreatic adenocarcinoma

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Background: Lactate dehydrogenase A (*LDHA*) plays a crucial role in the final step of anaerobic glycolysis, converting L-lactate and NAD^+ to pyruvate and nicotinamide adenine dinucleotide (NADH). Its high expression has been linked to tumorigenesis and patient survival in various human cancers. However, the full implications of *LDHA*'s role and its correlation with clinicopathological features in pancreatic adenocarcinoma (PAAD) remain to be fully understood. This study was thus conducted to elucidate the specific functions of *LDHA* in PAAD, with the aim of providing more robust evidence for clinical diagnosis and treatment.

Methods: In an extensive systems analysis, we searched through numerous databases, including The Cancer Genome Atlas (TCGA) and Oncomine. Our objective was to clarify the clinical implications and functional role of *LDHA* in PAAD. Bioinformatics was used to identify the biological function of *LDHA* expression and its correlation with tumor immune status.

Results: Our analysis revealed that the *LDHA* gene is overexpressed in PAAD and that this upregulation was associated with a worse patient prognosis. Through gene set enrichment analysis, we found that *LDHA*'s influence on PAAD is linked to signaling pathways involving Kirsten rat sarcoma viral oncogene homolog (*K-Ras*), transforming growth factor-β (*TGF-β*), and hypoxia inducible factor-1 (*HIF-1*). Mutation of *K-Ras* could upregulate its own expression and was positively correlated with *LDHA* expression. Moreover, our data demonstrated that *LDHA* expression was linked to immune infiltration and poor prognosis in PAAD, indicating its role in disease pathogenesis. Overexpression of *LDHA* may suppress tumor immunity, suggesting it as a potential target for the diagnosis and treatment of PAAD, thus providing new insights into managing this aggressive cancer.

Conclusions: Overall, our results showed that *LDHA* as a prognostic biomarker could serve as a novel target for future PAAD immunotherapy.

Keywords: Lactate dehydrogenase A (*LDHA*); biomarker; immune infiltrates; pancreatic adenocarcinoma (PAAD)

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Introduction

Pancreatic adenocarcinoma (PAAD), a lethal subtype of pancreatic cancer, is a significant global cause of cancer mortality, demanding urgent research for improved diagnosis and treatment strategies to address its aggressive nature (1,2). Despite advancements in surgical procedures and chemotherapy, PAAD remains a leading cause of cancer death in developed countries and across the globe (2). Currently, the most common treatments for PAAD include surgical resection, radiotherapy, and chemotherapy, yet patients may exhibit varying progression-free survival (PFS) and overall survival (OS) outcomes despite receiving the same treatment, likely due to individual responses to tumor immune infiltrates (3). Prognostic biomarkers are essential for identifying patients at high risk of recurrence following radical surgery, as these can enhance personalized treatment strategies for those with PAAD (4-6).

Under physiological oxygen conditions, pancreatic cancer cells predominantly rely on glycolysis for energy production, producing large amounts of lactic acid and small amounts of adenosine triphosphate, which is accompanied by glucose degradation, a phenomenon known as the Warburg effect (7). A study indicates tumor cells' metabolic reprogramming is key contributor to the resistance of tumor cells to chemoradiotherapy (8). Reversing the abnormal metabolism of tumor cells can significantly improve the sensitivity of patients to treatment, which would significantly prolong the survival of patients and improve their quality of life (9). In line with this, accumulating evidence suggests that the tumor microenvironment, reprogrammed metabolism glucose, amino acid and lipid metabolism, and metabolic crosstalk contribute to unrestrained pancreatic tumor progression (10). The regulation of the entire glycolysis process is primarily governed by the key enzymes *HK1*, *GAPDH*, pyruvate kinase isozyme type M2 (*PKM2*), and lactate dehydrogenase A (*LDHA*) (11-14).

LDHA is an important enzyme, as it is involved in the conversion of pyruvate to lactate and is a hallmark of aggressive cancers. Dysregulated expression of *LDHA* induces tumor cells to adopt lactate metabolism pathway, which is closely linked to tumor progression and treatment resistance. A hypoxic and acidic tumor microenvironment promote tumor immune escape while limiting the efficacy of immunotherapy (15). One study showed that overexpression *LDHA* is associated with poor prognosis in hepatocellular carcinoma (16), breast cancer (17), cholangiocarcinoma (18), papillary thyroid carcinoma (19), and pancreatic cancer and is correlated with a poor outcome. However, the pathological and clinical effects of *LDHA* expression and its immunological characteristics in PAAD still remain to be observed and investigated. Therefore, this study analyzed *LDHA* expression in PAAD using data from The Cancer Genome Atlas (TCGA) and investigated whether there is a correlation between *LDHA* expression and the progression of PAAD (20). Currently, immunotherapy provides limited efficacy for those with PAAD, and the underlying molecular mechanisms related its poor performance are unclear. Understanding the effects of *LDHA* in tumor energy metabolism and the immune microenvironment may improve the effectiveness of cancer immunotherapy (21-24). We present this article in accordance with the REMARK reporting checklist (available at https://jgo.amegroups.com/article/view/10.21037/jgo-24-560/rc).

Highlight box

Key findings

- Overexpression of lactate dehydrogenase A (*LDHA*) in pancreatic adenocarcinoma (PAAD) is linked to the tumor immune suppressive microenvironment.

What is Known and what is new?

- *LDHA* is overexpressed in a variety of malignant tumors and is an independent negative prognosis.
- *LDHA* overexpression in PAAD is associated with Kirsten rat sarcoma viral oncogene homolog (K-Ras) gene mutation status and glycolysis in PAAD tumor cells.

What is the implication, and what should change now?

- *LDHA* can be served as a potential intervention target for PAAD treatment.

Methods

Analysis of the Oncomine and TCGA datasets

Clinical information and gene expression data were

downloaded from TCGA (https://cancergenome.nih.gov/) and International Cancer Genome Consortium (ICGC; https://docs.icgc-argo.org/docs/data-access/daco/applying.) databases. The Oncomine database (http://www.oncomine.org) contains 264 independent datasets that include 35 cancer types and supports various methods of analysis. The expression of *LDHA* PPAD was investigated using the Human Protein Atlas (HPA) database (https://www.proteinatlas.org/). To avoid bias, another three independent patient populations from the ICGC portal database (http://bioinfo.henu.edu.cn/PAAD/PAADList.jsp) and EMTAB6314 were validated for *LDHA* expression and prognosis (19-28). Our study used open-source data that are freely available for research and publication and have no associated ethical concerns or other limitations. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Analysis of clinicopathological characteristics

RNA sequencing data from samples of 178 patients with PAAD and from adjacent tissues used as a normal control were obtained from TCGA. Corresponding clinicopathological data, including age, gender, pathological stage, tumor clinical stage, and OS, were also obtained from the database. Due to the missing of clinical data in some patients, the overall number of patients in each group was not completely consistent.

Survival analysis of LDHA expression in patients with PAAD

By analyzing the survival data of PAAD from TCGA database, we established a Cox proportional hazards model that considered all variables and their pairwise interactions. This model retained variables and interactions that were clinically significant and statistically significant ($P<0.05$) during the simplification process, allowing us to account for the complex interactions in the survival analysis of patients with PAAD. This model could potentially facilitate the precise treatment of individual patients and identify the dynamic factors affecting survival using unique tumor characteristics.

Functional enrichment analysis

The application of Gene Ontology (GO) frameworks allows researchers to systematically describe the functions of gene products across all forms of life. This approach provides a comprehensive understanding of the characteristic biological traits and transcript data derived from high-throughput genomic studies. In this analysis, we used the "ggplot2" package (version 3.3.3) and "cluster Profiler" package (version 3.14.3) in R (The R Foundation to Statistical Computing) to conduct a detailed examination of biological processes (BPs) and molecular functions (MFs).

Furthermore, we employed Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis to identify specific pathways that were differentially expressed between gene sets, providing higher-order functional insights that are essential for the functional interpretation and practical application of genomic information.

To clarify the potential BPs underlying the expression of *LDHA*, we conducted gene set enrichment analysis (GSEA). This analysis aimed to highlight the differences in BPs between groups with high and low *LDHA* expression levels, using a P value cutoff of <0.05 and a false discovery rate (FDR) of <0.25. To ensure the robustness of our findings, we set the number of permutations to 1,000, allowing for a reliable assessment of the significance of the observed differences.

Tumor immune microenvironment analysis

Single-sample GSEA via R package was used to calculate the proportional amounts of 22 invading immune cell types in each tumor sample (25). The ESTIMATE algorithm was used to deduce the immune scores for each sample. The relation of *LDHA* gene expression with immune infiltration was also analyzed with the Tumor Immune Estimation Resource 2.0 (TIMER2.0; http://timer.cistrome.org/). TIMER2.0 is a comprehensive resource for the systematic analysis of immune infiltrates across diverse cancer types.

Statistical analysis

All statistical analyses were conducted using GraphPad Prism version 13.0 (GraphPad Software, Inc., La Jolla, CA, USA) and R software (http://www.r-project.org/). Association coefficients were determined via the Pearson correlation method. Statistical comparisons among groups were conducted using the Wilcoxon rank-sum test or the Mann-Whitney test. Data are presented as the mean ± standard deviation. The prognostic significance of *LDHA* in pancreatic cancer was evaluated via receiver operating characteristic (ROC) curves and the area under the ROC

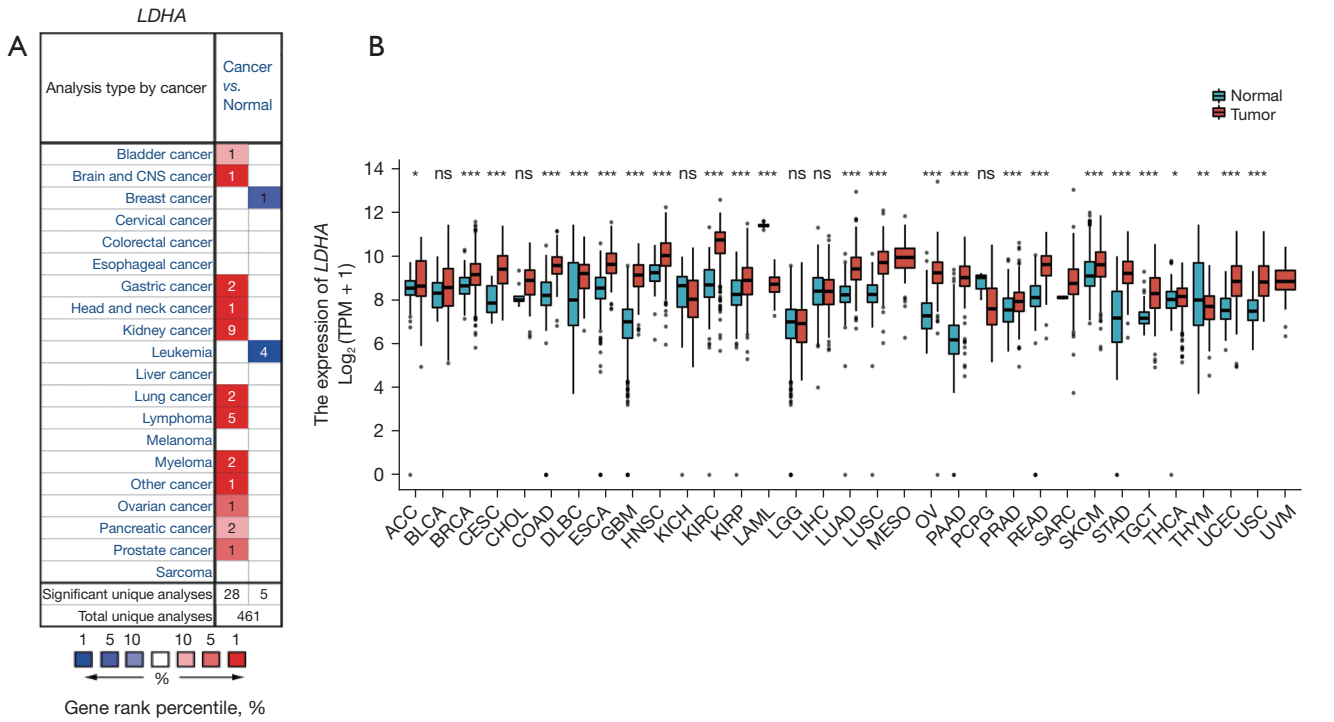

Figure 1 *LDHA* expression levels in different types of human cancers. (A) Increased or decreased *LDHA* in the datasets of various cancers compared with normal tissues in the Oncomine database. (B) Human *LDHA* expression levels in different tumor types from TCGA database were determined via TIMER. *, P<0.05; **, P<0.01; ***, P<0.001, ns: no statistical difference between two sets of data when compared. *LDHA*, lactate dehydrogenase A; CNS, central nervous system; TPM, transcripts per million; ns, not significant.

curve (AUC). The relationship between *LDHA* expression and patient clinicopathological characteristics was assessed via the Chi-squared test. Both univariate and multivariate survival analyses were performed employing the Cox proportional hazards regression model. Variables identified as significant in the univariate analysis were subsequently included in the multivariate survival analysis. Statistical significance was determined at a P value less than 0.05.

Results

Overexpression of LDHA in various malignancies

The details of the *LDHA* expression analyses are summarized in *Figure 1* and include the ROC curves for the ability of *LDHA* expression to identify tumor and normal tissue (*Figure 2A*). Additionally, the protein expression information from the HPA database indicated that *LDHA* was highly expressed in PAAD specimens at the protein level as compared to its expression in normal controls (https://www.proteinatlas.org/ENSG00000134333-LDHA/pathology/pancreatic+cancer#img). Unfortunately, the protein expression data for *LDHA* in the adjacent normal tissues were not available in this database. The ROC curve also showed that *LDHA* was highly expressed in tumor tissues, while the expression was relatively low in adjacent normal tissues [AUC =0.95; 95% confidence interval (CI): 0.92–0.97] (*Figure 2A*).

The relationship between *LDHA* expression and clinicopathological characteristics in PAAD was analyzed with TCGA database (*Table 1*). There were no significant relationships between *LDHA* expression and lymph node or distance metastasis. However, the high expression of *LDHA* correlated significantly with age and T stage (P<0.05).

High LDHA expression was associated with poor prognosis

To investigate the relationship between LDHA expression and prognosis in patients with PADD, we collected four independent databases (EMTAB6134, TCGA, ICGC-sequence and ICGC-array group). According to the

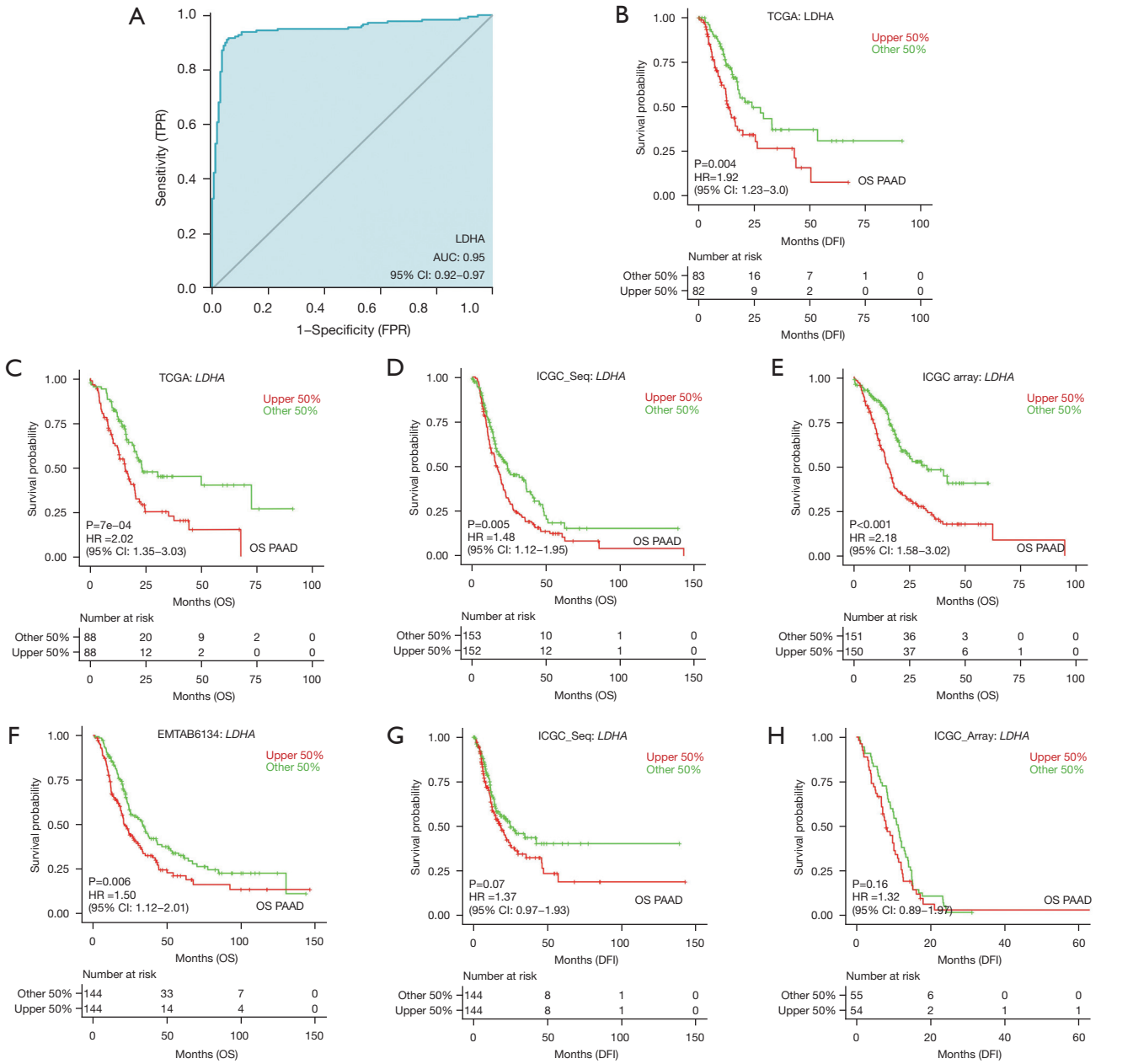

Figure 2 *LDHA* expression was high in tumor tissue and was associated with clinical prognosis. (A) ROC curve of *LDHA* high expression in tumor tissue specifically (AUC =0.95; 95% CI: 0.92–0.97; P<0.001). (B-E) Kaplan-Meier survival curves comparing the high and low expression of *LDHA* in OSpaad databases. (F-H) Survival curves of DFI in three independent PAAD cohorts. TPR, true positive rate; FPR, false positive rate; *LDHA*, lactate dehydrogenase A; AUC, area under the curve; HR, hazard ratio; CI, confidence interval; OS, overall survival; PAAD, pancreatic adenocarcinoma; DFI, disease-free interval; TCGA, The Cancer Genome Atlas; ICGC, International Cancer Genome Consortium; ROC, receiver operating characteristic; IHC, immunohistochemistry.

Table 1 Clinical characteristics of the patients with pancreatic cancer

Characteristic	Low expression of LDHA (n=89)	High expression of LDHA (n=89)	P value*
T stage			0.02
T1 + T2	21 (11.9)	10 (5.7)	
T3 + T4	66 (37.5)	79 (44.9)	
N stage			0.19
N0	30 (17.3)	20 (11.6)	
N1	56 (32.4)	67 (38.7)	
M stage			0.67
M0	37 (44)	42 (50.0)	
M1	3 (3.6)	2 (2.4)	
Age (years)	66.36±9.71	63.13±11.62	0.046

Note: some of the patient's data parameters were missing. Data are expressed as n (%) or mean ± standard deviation. *, compared via the Pearson Chi-squared test or Fisher exact test. LDHA, lactate dehydrogenase A.

median expression level of *LDHA*, patients were stratified into two groups: low and high expression. Kaplan-Meier survival analysis revealed that patients with higher *LDHA* expression levels experienced a reduced PFS rate [hazard ratio (HR) =1.92, 95% confidence interval (CI): 1.23–3.0; P=0.004; *Figure 2B*] and poorer OS (HR =2.02, 95% CI: 1.34–3.03; P<0.001; *Figure 2C*). Univariate Cox regression analysis demonstrated a significant correlation between the upregulation of *LDHA* and adverse PFS and OS (P<0.001; *Tables 2,3*) (26). Other clinical features, including N stage, T stage, histologic grade, primary therapy outcome, and residual tumor were also found to be associated with poor PFS and OS (P<0.05). Moreover, multivariate Cox proportional hazard regression analysis demonstrated that high *LDHA* expression (P<0.001 for PFS and OS) and primary therapy outcome and residual tumor (P<0.05 for OS and PFS) were independent risk factors for poor prognosis. Radiation therapy and lower histological grade served as an independent predictor of better OS (P<0.05); however, they were not associated with PFS. To avoid patient population bias, three additional independent samples (EMTAB6134, ICGC_Seq, and ICGC_array) were included and analyzed with the OSpaad program (27), which confirmed the high expression of *LDHA* to be significantly associated with OS (ICGC_Seq: HR =1.48, 95% CI: 1.12–1.95, P=0.005; ICGC_array: HR =2.18, 95% CI: 1.58–3.02, P<0.001; EMTAB6134: HR =1.50, 95% CI: 1.12–2.01, P=0.006) (*Figure 2D-2F*), but non-significantly associated with PFS (HR =1.37, 95% CI: 0.97–1.93, P=0.07; HR =1.32, 95% CI: 0.89–1.97, P=0.16) (*Figure 2G,2H*). Collectively, these results show that *LDHA* expression may be a prognostic biomarker of PAAD.

GO and KEGG enrichment analyses

The GO enrichment analysis indicated that high *LDHA* expression was linked with the hypoxia inducible factor-1 (HIF-1) signaling pathway, glycolysis, coenzyme binding, and the pyruvate and nicotinamide nucleotide and pyridine nucleotide metabolic processes (*Figure 3A*). Cell composition enrichment analysis indicated *LDHA* expression was closely related with cell-cell junction, cell-substrate junction, cell-substrate adherent junction, collagen-containing extracellular matrix, and focal adhesion (*Figure 3B*). These results suggest that the *LDHA* gene is involved in anaerobic fermentation and tumor energy and material metabolism. A high expression of *LDHA* can remodel the connection between cells and extracellular matrix, which may lead to tumor invasion and distant metastasis.

LDHA expression was correlated with activation of the glycolysis, HIF-1, and Kirsten rat sarcoma viral oncogene homolog (K-Ras) pathways

GSEA revealed that the *LDHA* gene was significantly enriched in glycolysis [normalized enrichment score (NES) =1.84; adjusted P value (P.adjust) =0; FDR =0.002;

Table 2 Univariate and multivariate analysis of progression-free survival in patients with pancreatic cancer

Characteristics	Total (N)	Univariate analysis		Multivariate analysis	
		HR (95% CI)	P value	HR (95% CI)	P value
T stage (T3 + T4 *vs.* T1 + T2)	176	2.414 (1.309–4.452)	0.005*	1.282 (0.667–2.466)	0.45
N stage (N1 *vs.* N0)	173	1.735 (1.113–2.705)	0.015*	1.492 (0.886–2.513)	0.13
Pathologic stage (stage III–IV *vs.* stage I–II)	175	1.109 (0.484–2.540)	0.81	–	–
M stage (M1 *vs.* M0)	84	0.837 (0.300–2.336)	0.73	–	–
Primary therapy outcome (PR + CR *vs.* PD + SD)	139	2.975 (1.908–4.640)	<0.001*	2.113 (1.303–3.426)	0.002*
Gender (male *vs.* female)	178	0.968 (0.658–1.423)	0.87	–	–
Radiation therapy (yes *vs.* no)	163	0.744 (0.474–1.168)	0.20	–	–
Residual tumor (R1 + R2 *vs.* R0)	164	2.253 (1.494–3.398)	<0.001*	1.968 (1.203–3.219)	0.007*
Age (>65 *vs.* ≤65 years)	178	1.256 (0.848–1.861)	0.26	–	–
Histologic grade (G3 + G4 *vs.* G1 + G2)	176	1.684 (1.114–2.546)	0.01*	1.562 (0.964–2.531)	0.07
Smoker (yes *vs.* no)	144	1.048 (0.683–1.606)	0.83	–	–
Alcohol history (yes *vs.* no)	166	1.217 (0.799–1.851)	0.36	–	–
History of diabetes (yes *vs.* no)	146	0.783 (0.460–1.333)	0.37	–	–
History of chronic pancreatitis (yes *vs.* no)	141	0.885 (0.426–1.840)	0.74	–	–
LDHA (high *vs.* low)	178	2.250 (1.516–3.339)	<0.001*	2.081 (1.282–3.377)	0.003*

*, statistically significant P value <0.05. HR, hazard ratio; CI, confidence interval; PR, partial response; CR, complete response; PD, progressive disease; SD, stable disease; LDHA, lactate dehydrogenase A.

Table 3 Univariate and multivariate analysis of LDHA expression on overall survival in patients with pancreatic cancer

Characteristics	Total (N)	OS			
		Univariate analysis		Multivariate analysis	
		HR (95% CI)	P value	HR (95% CI)	P value
T stage (T3 + T4 *vs.* T1 + T2)	176	2.023 (1.072–3.816)	0.03*	1.139 (0.554–2.344)	0.72
N stage (N1 *vs.* N0)	173	2.154 (1.282–3.618)	0.004*	1.728 (0.912–3.273)	0.09
Pathologic stage (stage III–IV *vs.* stage I–II)	175	0.673 (0.212–2.135)	0.50		
M stage (M1 *vs.* M0)	84	0.756 (0.181–3.157)	0.70		
Primary therapy outcome (PR + CR *vs.* PD + SD)	139	0.425 (0.267–0.677)	<0.001*	0.526 (0.315–0.877)	0.01*
Gender (male *vs.* female)	178	0.809 (0.537–1.219)	0.31		
Radiation therapy (yes *vs.* no)	163	0.508 (0.298–0.866)	0.01*	0.514 (0.270–0.981)	0.04*
Residual tumor (R1 + R2 *vs.* R0)	164	1.645 (1.056–2.561)	0.03*	1.765 (1.026–3.038)	0.04*
Age (>65 *vs.* ≤65 years)	178	1.290 (0.854–1.948)	0.22		
Histologic grade (G3 + G4 *vs.* G1 + G2)	176	1.538 (0.996–2.376)	0.05*	1.792 (1.042–3.080)	0.03*
Smoker (yes *vs.* no)	144	1.086 (0.687–1.719)	0.72		
Alcohol history (yes *vs.* no)	166	1.147 (0.738–1.783)	0.54		
History of diabetes (yes *vs.* no)	146	0.927 (0.532–1.615)	0.79		
History of chronic pancreatitis (yes *vs.* no)	141	1.177 (0.562–2.464)	0.67		
LDHA (high *vs.* low)	178	2.566 (1.668–3.948)	<0.001*	1.911 (1.120–3.260)	0.01*

*, statistically significant P value <0.05. LDHA, lactate dehydrogenase A; OS, overall survival; HR, hazard ratio; CI, confidence interval; PR, partial response; CR, complete response; PD, progressive disease; SD, stable disease.

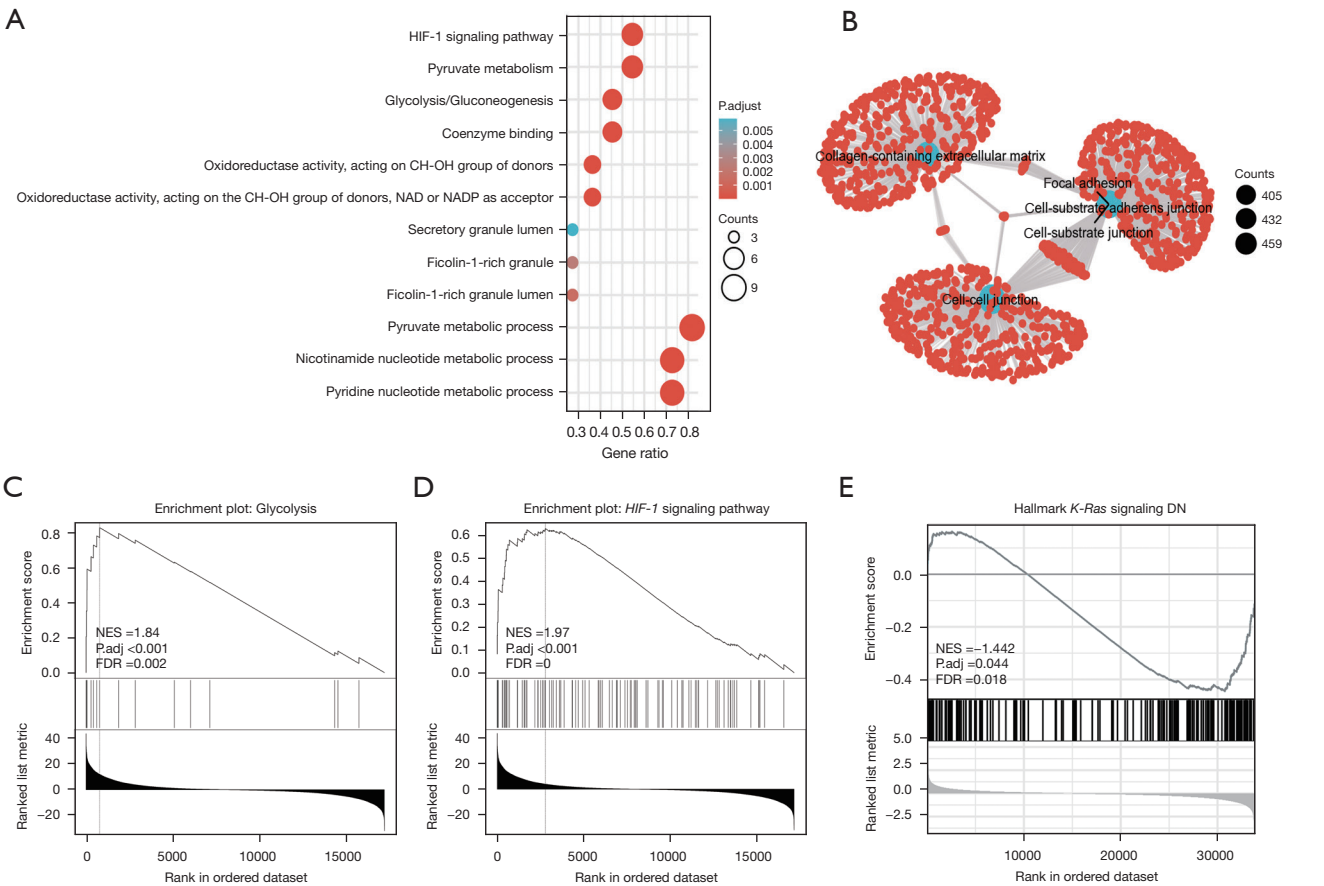

Figure 3 Functional enrichment analysis of DEGs in TCGA-PAAD patients. (A,B) GO enrichment analysis of the target module genes, biological process analysis, and cellular component analysis. The y-axis represents the enriched KEGG terms. The x-axis represents the fold of enrichment. The size of the dot represents the number of genes under a specific term, and the color of the dots represents the adjusted P value. (C-E) *HIF-1*, *TGF-β*, and *K-Ras* gene sets with statistically significant differences in GSEA analysis. HIF-1, hypoxia inducible factor-1; NES, normalized enrichment score; P.adj, adjusted P value; FDR, false discovery rate; DN, down-regulation; DEGs, differentially expressed genes; TCGA, The Cancer Genome Atlas; PAAD, pancreatic adenocarcinoma; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; GSEA, gene set enrichment analysis; K-Ras, Kirsten rat sarcoma viral oncogene homolog; TGF-β, transforming growth factor-β.

Figure 3C], *HIF-1* (NES =1.97; P.adjust <0.001; FDR =0; *Figure 3D*), and *K-Ras* pathway (NES =–1.442; P.adjust = 0.04; FDR =0.018; *Figure 3E*). Indeed, we found that there was a positive correlation between *LDHA* and transforming growth factor-β (TGF-β) pathway activation (*Figure 4A*,*4B*) and the *K-Ras* gene mutation upregulated its own expression (*Figure 4C*), and the expression level of *LDHA* was positively correlated with *K-Ras* gene expression and mutation status (*Figure 4D*). Similarly, these findings indicate that *LDHA* plays a crucial role in tumor cell metabolic reprogramming.

Relationship between LDHA expression and the tumor immune microenvironment

The immune status and cell infiltration associated with *LDHA* expression was analyzed (*Figure 5A*). After adjustments were made according to purity, the T helper 2 (Th2) cell subpopulation was found to be positively correlated with *LDHA* expression, while most immune cells, including dendritic cells (DCs), interstitial DCs (iDCs), $CD8^+$ T cells, B cells, T cells, and plasmacytoid DCs

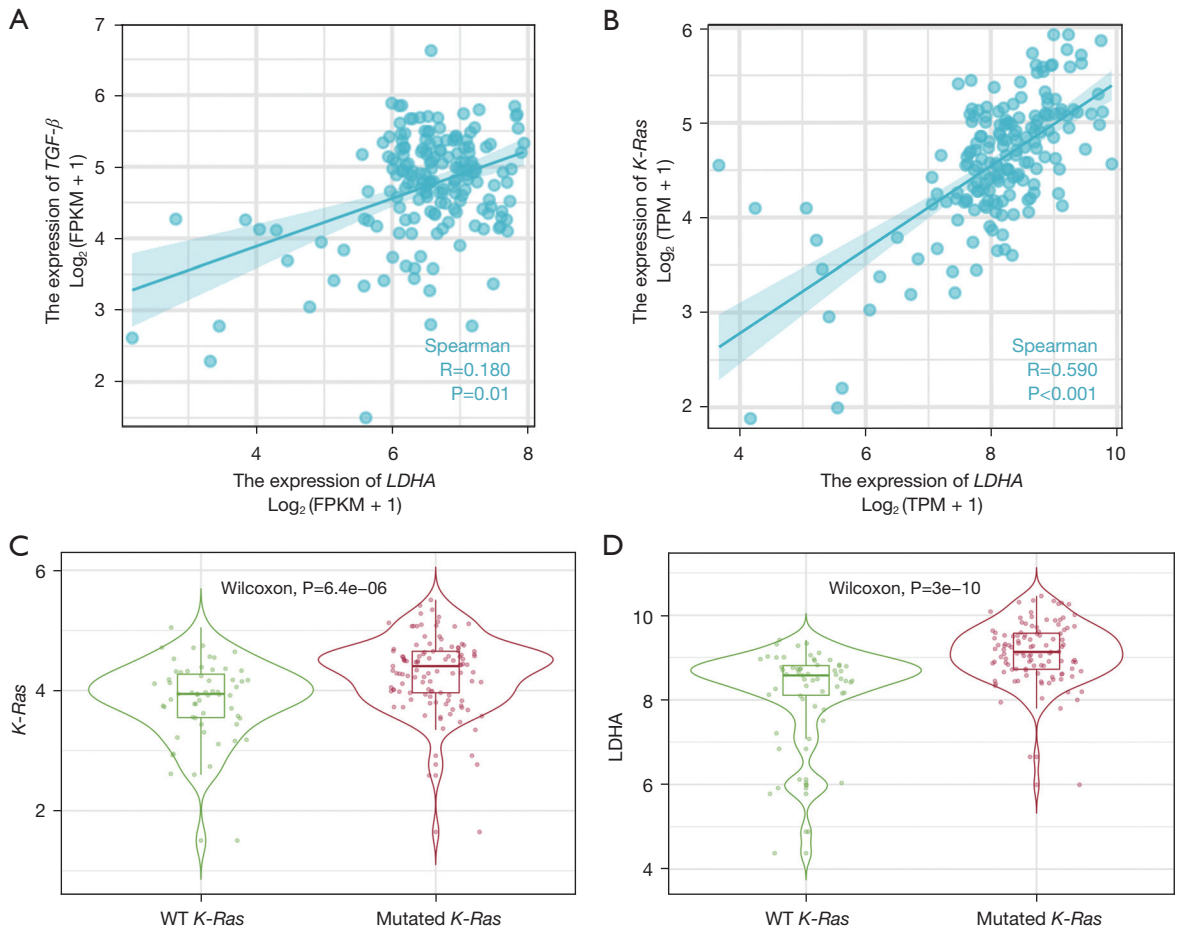

Figure 4 *LDHA* expression was positively correlated with the activation of the *TGF-β* and *K-Ras* mutation pathways. (A,B) Pearson correlation of *LDHA* and *TGF-β* and *K-Ras* expression. Both genes had a significant correlation (P<0.05). The x-axis represents the expression level of *LDHA*, and the y-axis represents the expression level of *TGF-β* and *K-Ras* in TCGA-PAAD patients. (C) Mutation of the *K-Ras* gene upregulated its own mRNA expression level (P<0.001). (D) Patients with *K-Ras* mutation showed higher *LDHA* mRNA expression (P<0.001). TGF-β, transforming growth factor-β; *LDHA*, lactate dehydrogenase A; FPKM, fragments per kilobase of transcript per million fragments mapped; TPM, transcripts per million; WT, wild type; TCGA, The Cancer Genome Atlas; PAAD, pancreatic adenocarcinoma.

(pDCs) were negatively correlated with gene expression. The immune score of tumors was negatively correlated with *LDHA* expression (P<0.05; *Figure 5B*). After the correlation was adjusted according to purity, the results revealed the *LDHA* expression level had significant positive correlations with infiltrating levels of $CD4^+$ Th2 cells (r=0.282; P=1.87e−04), M1 macrophages (r=0.401; P=5.53e−08), and myeloid-derived suppressor cells (MDSCs) (r=0.568; P=5.25e−16). Meanwhile, *LDHA* expression was negatively correlated with $CD4^+$ memory T cells (r=−0.153; P=4.53e−02), $CD8^+$ effector memory T cells (r=−0.185; P=1.55e−02, class-switched memory B cells (r =−0.19; P=1.27e−02), M2 macrophages (r=−0.283; P=1.78e−04), and natural killer (NK) T cells (r=−0.155; P=4.34e−2). Finally, *LDHA* expression showed no significant correlations with tumor purity or infiltration levels of $CD4^+$ Th1 (r=−0.004; P=9.61e−01) (*Figure 5C*). These results strongly suggest that *LDHA* plays a certain role in immune infiltration in PAAD and may contribute to the limited curative effect of immunotherapy and poor prognosis.

Discussion

The current treatments for pancreatic cancer are not

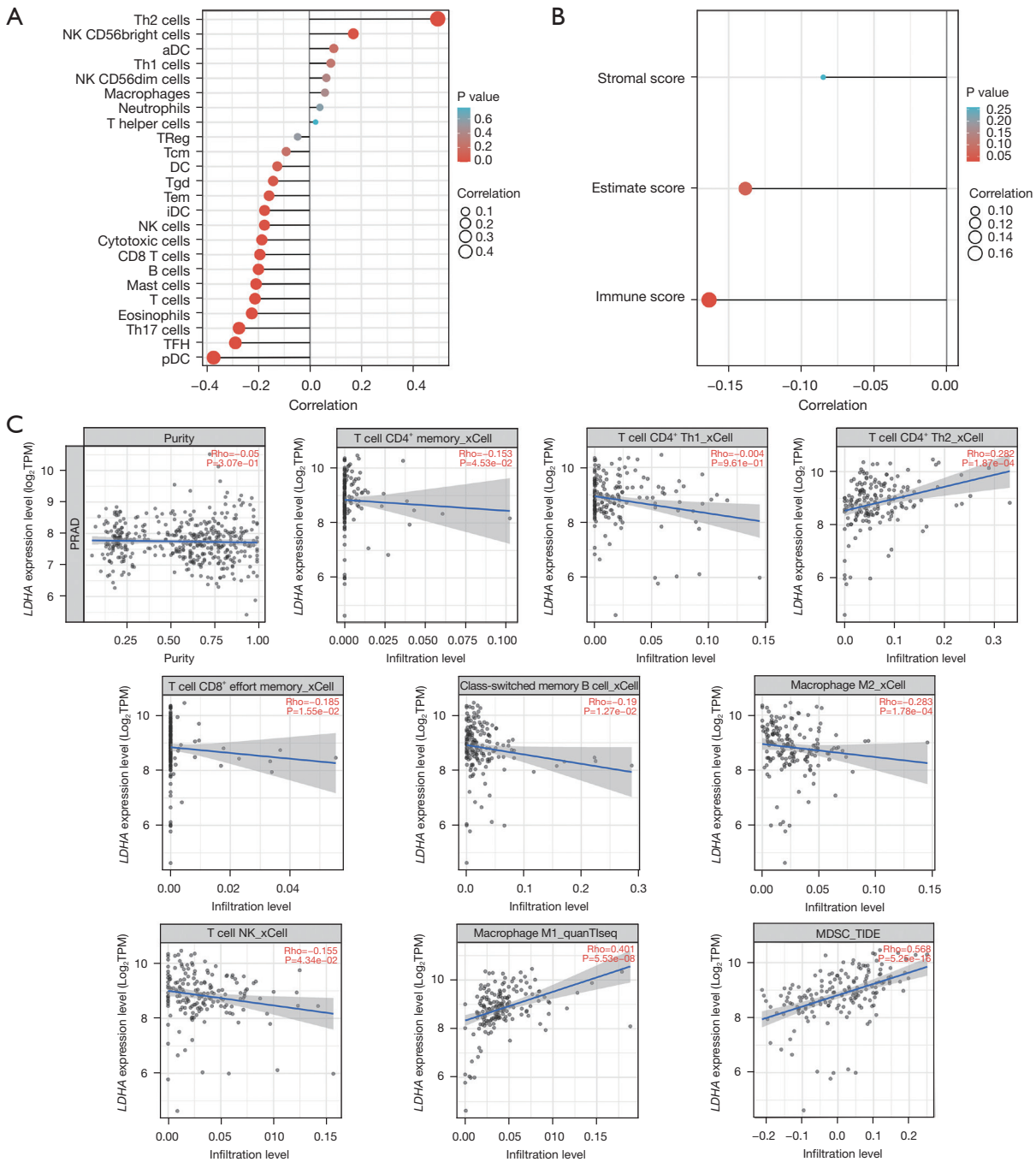

Figure 5 Correlation of *LDHA* expression with immune depression status in patients with PAAD. (A) *LDHA* expression demonstrated a significantly positive correlation with the infiltration levels of Th2 T cells and CD56 bright NK cells and negatively correlations with NK cells, cytotoxic T cells, $CD8^+$ T cells, B cells T cells, Th17 cells, and pDCs (n=179). (B) *LDHA* expression demonstrated a significantly negative correlation with tumor ESTIMATE score and immune score. (C) *LDHA* expression demonstrated a significantly positive correlation with $CD4^+$ Th2 cells, M1 macrophages and MDSC, and a significantly negative correlation with the infiltration levels of $CD4^+$ memory T cells, $CD8^+$ effector memory T cells, class-switched memory B cells, M2 macrophages, and NK T cells. Th, T helper; NK, natural killer; aDC, activated dendritic cell; TReg, regulatory T cell; Tcm, central memory T cell; DC, dendritic cell; Tgd, gamma delta T cell; Tem, effector memory T cell; iDC, immature dendritic cell; TFH, follicular helper T cell; pDC, plasmacytoid dendritic cell; TPM, transcripts per million; *LDHA*, lactate dehydrogenase A; PAAD, pancreatic adenocarcinoma; MDSC, myeloid-derived suppressor cell.

satisfactory, with the efficacy of immunotherapy being particularly poor. Cancer metabolism is heavily reliant on the dysregulation of enzymatic pathways, with oxidative glycolysis remaining a prominent characteristic (28,29). In this study, we observed an increased expression of *LDHA* in multiple types of cancerous tumors, encompassing PAAD. The ROC curve analysis indicated that *LDHA* may have strong diagnostic ability in differentiating healthy individuals from those with PAAD, thus highlighting the pivotal function of *LDHA* in the process of cancerous transformation (15,30) and its potential as a target for treatment.

Immunosuppression in cancer has become a significant challenge to the efficacy of immunotherapeutic strategies and stems from both oncogene-induced signaling within the tumor and immune cells associated with the tumor. Nevertheless, the involvement of *LDHA* in the onset and advancement of PAAD remains inadequately clarified. *LDHA* fosters the aggressive development of cancer by enhancing lactate generation, accelerating glucose absorption, and controlling various cancer-related molecules, making it a crucial enzyme in the regulation of anaerobic glycolysis (31-34). To determine the correlation between the levels of *LDHA* expression and the progression of PAAD, we analyzed datasets from the Oncomine database and TCGA. TCGA database was employed to examine the pathological attributes and prognosis associated with *LDHA* expression (22). It was found that the abnormal expression of *LDHA* was related to the age of patients, and a prognostic model was subsequently constructed to a provide basis for clinical evaluation of patient prognosis. Through enrichment analysis, it was found that *LDHA* was related to *K-Ras* pathway, and the expression of *LDHA* was positively correlated with *K-Ras*. *K-Ras* gene mutation could upregulate the expression of *LDHA*.

The concomitant expression of *SIP1* and *LDHA* might indicate the presence of both epithelial-mesenchymal transition and the Warburg effect in the progression of PAAD. Additionally, we observed that the levels of *LDHA* expression were significantly linked to patient age. Survival analysis revealed that the higher expression of *LDHA* was associated with a significantly reduced OS. In our investigation, regression analyses highlighted a stronger correlation between *LDHA* expression and clinical outcome. Although these findings may seem counterintuitive, they are statistically valid but could be attributed to the limited sample size, and thus further validation is required. Collectively, our data suggest that *LDHA* expression serves as an independent prognostic factor for OS in patients with PAAD.

K-Ras is a Ras protein and plays a key role in cancer. Enabling somatic *K-Ras* mutations are linked to more than 15% of all human cancers, and their incidence can reach as high as 90% in certain tumor categories, including PAAD. Consequently, effectively blocking the abnormal *K-Ras* signaling pathway would constitute a paradigm shift in cancer treatment (35). Previous research suggests that the expression levels underlying the metabolic alterations in oncogenic *K-Ras* conversion can be attributed to the modifications in the activity of key enzymes in NIH-3T3 cells. These modifications, along with the metabolic shifts following oncogenic transformation—including heightened glycolysis, activation of the oxidative arm of the pentose-phosphate pathway, and reduced levels of sugar phosphates—might be correlated with the enhanced activity of glucose-6-phosphate dehydrogenase, pyruvate kinase, and lactate dehydrogenase as well as the diminished activity for transketolase (36). *K-Ras* gene polymorphisms are associated with susceptibility to non-Hodgkin lymphoma and gene expression and levels of *LDHA* (37). In our study, through enrichment analysis, it was found that *LDHA* was related to the *K-Ras* pathway, and the expression of *LDHA* was positively correlated with *K-Ras*. *K-Ras* mutation may upregulate the expression of *LDHA* and play an important role in PAAD carcinogenesis and prognosis. Thus, the relationship of *LDHA* with the *K-Ras* pathway warrants further investigation.

Immune cells are critically involved in cancer progression and treatment, and the tumor immune microenvironment exerts significant immunologically relevant changes by inducing severe immune-mediated toxicities (38). Overexpression of LDHA is closely associated with aberrant activation of the *K-Ras* gene pathway, glycolysis, and HIF-1, indicating that tumor cells undergo metabolic reprogramming (39). This results in a negative immune microenvironment and tumor immunosuppression, ultimately leading to poor prognosis for patients. Most glycolytic inhibitors not only inhibit the glycolysis of cancer but also that of immune cells (40).

Serganova *et al.* reported that reduction in *LDHA* inhibits the development of metastases and extends the survival of mice by altering the tumor microenvironment, which in turn regulates the immune response (41). In our study, DCs, iDCs, $CD8^+$ T cells, B cells, T cells, and

pDCs were negatively correlated with *LDHA* expression, which is consistent with and can be explained by previous studies (42-44). Although our study suggested no significant correlation between LDHA expression and total neutrophil count (as shown in *Figure 5*), Wang *et al.* (10) demonstrated a positive correlation with cancer-promoting neutrophil subtypes through single gene sequencing. The discrepancy in findings may stem from variations in research methodologies, which could be further elucidated through single cell sequencing in subsequent studies. The overexpression of LDHA in PAAD may interact with immune cell mechanisms in the following ways (45,46): firstly, it can promote glycolytic metabolism and generate lactic acid to fuel tumor energy needs, thereby influencing immune cells through changes in the microenvironment. Secondly, it can contribute to an immunosuppressive microenvironment by directly inhibiting or inducing immunosuppressive cells and weakening anti-tumor responses. Lastly, metabolites such as lactic acid may impact the metabolism and function of immune cells through specific pathways, leading to inhibition of activation and proliferation. The optimal therapeutic agents are capable of destroying tumor cells and remodeling the tumor immune microenvironment, conferring both anticancer and immunostimulatory effects (47-49).

There are certain limitations related to this study which should be noted. First, due to the retrospective nature of the analyses, a certain degree of selection bias was unavoidable. Second, all the laboratory metrics were confined to preoperative assessments, and additional data from prospective trials are needed to validate these findings. Third, it should be noted that this study was purely observational in design, and thus it is necessary to devise animal experiments to further examine the effect of *LDHA* intervention on tumor metabolism and tumor immune microenvironment remodeling *in vivo*.

Conclusions

In summary, *LDHA* expression was increased in PAAD tissue, which was related to patient prognosis and immune infiltration. Furthermore, elevated expression of *LDHA* was linked to activation of the *K-Ras* signaling pathway and the presence of immune cell populations in PAAD. Consequently, *PDCD1* could function as a predictive biomarker for PAAD. Our findings may lead to the development of an immunologically targeted antitumor approach that encompasses metabolic rewiring of either the tumor cells themselves or the immune cells infiltrating the tumor microenvironment.

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Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://jgo.amegroups.com/article/view/10.21037/jgo-24-560/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jgo.amegroups.com/article/view/10.21037/jgo-24-560/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).

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