Research Paper

HLA-DPA1 overexpression inhibits cancer progression, reduces resistance to cisplatin, and correlates with increased immune infiltration in lung adenocarcinoma

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ABSTRACT

Purpose: Human Leukocyte Antigen-DP alpha 1 (HLA-DPA1) is a critical gene in antigen-presenting cells and plays a significant role in immune regulation. The objective of this study was to comprehensively analyze the roles of HLA-DPA1 and its association with lung adenocarcinoma (LUAD).

Methods: We utilized bioinformatics and conducted a meta-analysis to examine the roles of HLA-DPA1 expression on the progression and immunity of LUAD. We also performed CCK-8, wound healing, and Transwell assays to validate the functions of HLA-DPA1 in LUAD.

Results: HLA-DPA1 expression is downregulated in LUAD tissues and is associated with gender, race, age, smoking history, clinical stage, histological type, lymph node metastasis, and prognosis of patients with LUAD. HLA-DPA1 is involved in immune responses, leukocyte cell-cell adhesion, and antigen processing and presentation. Overexpression of HLA-DPA1 inhibits cancer cell proliferation, migration, and invasion while promoting cell sensitivity to cisplatin in A549 and A549/DDP cells. Additionally, overexpression of HLA-DPA1 correlates with tumor purity, stromal, immune, and ESTIMATE scores, the abundance of immune cells (B cells, $CD8^+$ T cells, $CD4^+$ T cells, macrophages, dendritic cells, and neutrophils), and immune cell markers (programmed cell death 1, cytotoxic T-lymphocyte-associated protein 4, and cluster of differentiation 8A).

Conclusions: Decreased HLA-DPA1 expression is associated with poor prognosis and immune infiltration in LUAD while HLA-DPA1 overexpression inhibits cancer cell proliferation and progression. Therefore, HLA-DPA1 shows potential as a prognostic biomarker and a therapeutic target for LUAD.

INTRODUCTION

Lung cancer is a globally prevalent malignancy with one of the highest mortality rates among cancers [1]. Lung adenocarcinoma (LUAD) constitutes one of the most common subtypes of lung cancer [2, 3]. The immune system plays a pivotal role in tumorigenesis and cancer treatments. Immunotherapy has recently emerged as a promising therapeutic option for lung cancer [4–6]. For instance, programmed cell death

protein 1 (PD-1) positive tumors lead to increased levels of T cells and programmed cell death ligand 1 (PD-L1) expression. Decreasing PD-1 expression helps control tumor growth, improves overall survival rates among cancer patients, and contributes to the reprogramming of tumor-associated lymph and myeloid cells [5]. A low-dose of apatinib, a small molecule tyrosine kinase inhibitor, alleviates hypoxia, increases cluster of differentiation (CD)8^+ T cell infiltration, and reduces tumor-related macrophage recruitment in murine lung cancer models. Combining it with anti-PD-L1 effectively slows tumor growth, decreases cancer metastasis, and prolongs survival time. This combination can also enhance anticancer activity against advanced lung cancer [6]. Identifying new prognostic and immune-related targets for the progression of lung cancer represents a crucial need.

The major histocompatibility complex (MHC), DP alpha 1 (DPA1), also known as HLA-DPA1, located on chromosome 6p21.3, plays a critical role in the functioning of antigen-presenting cells, which are related to immune regulation. HLA-DPA1 is abnormally expressed in cancer tissues, contributing to cancer progression [7, 8]. For instance, downregulation of HLA-DPA1 expression is significantly associated with poor prognosis of adults with adrenocortical tumors, being an independent prognostic factor for these patients [7]. Similarly, HLA-DPA1 downregulation in multiple myeloma is correlated with disease-specific survival (DSS) of the affected patients [8]. However, the relationship between HLA-DPA1 expression, patient prognosis, and LUAD progression remains unclear. This study comprehensively explored the role of HLA-DPA1 in LUAD progression and the underlying pathways, aiming to identify a novel target for LUAD treatment.

RESULTS

Expression of HLA-DPA1 in LUAD tissues

Based on data retrieved from the Gene Expression Omnibus (GEO) database, we found that HLA-DPA1 levels were significantly lower in LUAD tissues compared to normal tissue controls (Figure 1). The samples included 86 LUAD tissues vs. 10 normal tissues, 58 LUAD tissues vs. 58 normal tissues, 58 LUAD tissues vs. 49 normal tissues, and 20 LUAD tissues vs. 19 normal tissues from the GSE68751, GSE32863, GSE10072, and GSE2514 datasets, respectively (Figure 1A–1D). These trends were consistent when analyzing the data retrieved from the UALCAN database (Figure 2A).

HLA-DPA1 expression correlates with the clinical indicators in patients with LUAD

Using data from the UALCAN database, we observed that decreased levels of HLA-DPA1 were associated with several demographic and clinical factors in patients with LUAD. These factors included gender, race, age, smoking history, clinical stage, histological subtype, and lymph node metastasis (Figure 2B–2H). Specifically, we found that HLA-DPA1 expression was lower in males compared to females, and in Caucasians compared to African Americans. Additionally, specific age ranges and smoking history were associated with decreased expression levels of HLA-DPA1. We also observed correlations between low HLA-DPA1 expression and the clinical stages, histological subtype, and lymph node metastasis categories in patients with LUAD ($P < 0.05$).

HLA-DPA1 expression correlates with LUAD diagnosis and prognosis

Using data from both the Cancer Genome Atlas (TCGA) and XENA databases, we calculated that area under the curve for HLA-DPA1 in normal and cancer tissues were 0.86 and 0.842, respectively (Figure 3A, 3B). Our findings suggest that HLA-DPA1 has significant diagnostic significance. Moreover, our study revealed that a decrease in HLA-DPA1 expression was associated with poor prognosis, overall survival (OS), DSS, and progression-free interval (PFI), in patients with LUAD (Figure 3C–3E). Additionally, a meta-analysis of data within the Lung Cancer Explorer (LCE) database also demonstrated that decreased expression of HLA-DPA1 was related to poor OS for patients with LUAD (Figure 4).

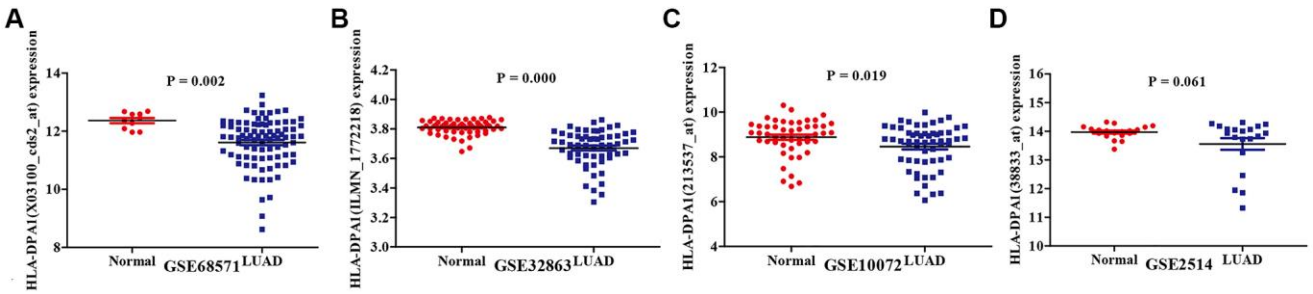

Figure 1. HLA-DPA1 expression in lung adenocarcinoma tissues. (**A**) GSE68751; (**B**) GSE32863; (**C**) GSE10072; (**D**) GSE2514.

HLA-DPA1 roles and underlying mechanisms as revealed by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses

Our analysis of LUAD tissues revealed 229 genes that were positively co-expressed with HLA-DPA1 based on correlation coefficients (Figure 5 and Supplementary Table 1). These co-expressed genes participate in various biological processes, including the MHC class II protein complex-mediated immune response, inflammatory response, positive regulation of T cell activation, cell surface functions, antigen processing and presentation, adaptive immune response, MHC class II receptor activity, as well as positive regulation of interferon-gamma production, tumor necrosis factor production, and T-cell mediated cytotoxicity. Furthermore, these genes are expressed in various signaling pathways, including cell adhesion molecules, Toll-like receptor, B cell receptor, Rap1, and chemokine signaling pathways, as well as Th1, Th2, and Th17 cell differentiation, natural killer cell-mediated cytotoxicity, and cytokine-cytokine receptor interaction, as revealed through GO and KEGG analyses (Supplementary Table 2 and Table 1). Utilizing the Tumor-Immune System Interaction Database (TISIDB), we revealed that HLA-DPA1 was involved in several biological processes, including the positive regulation of cytokine production, immune response, antigen processing and presentation, leukocyte cell-cell adhesion, T cell co-stimulation, proliferation, activation, and receptor signaling pathway, leukocyte proliferation, and presentation pathways, including cell adhesion molecules, antigen processing and presentation, and hematopoietic cell lineage pathways (Supplementary Table 3).

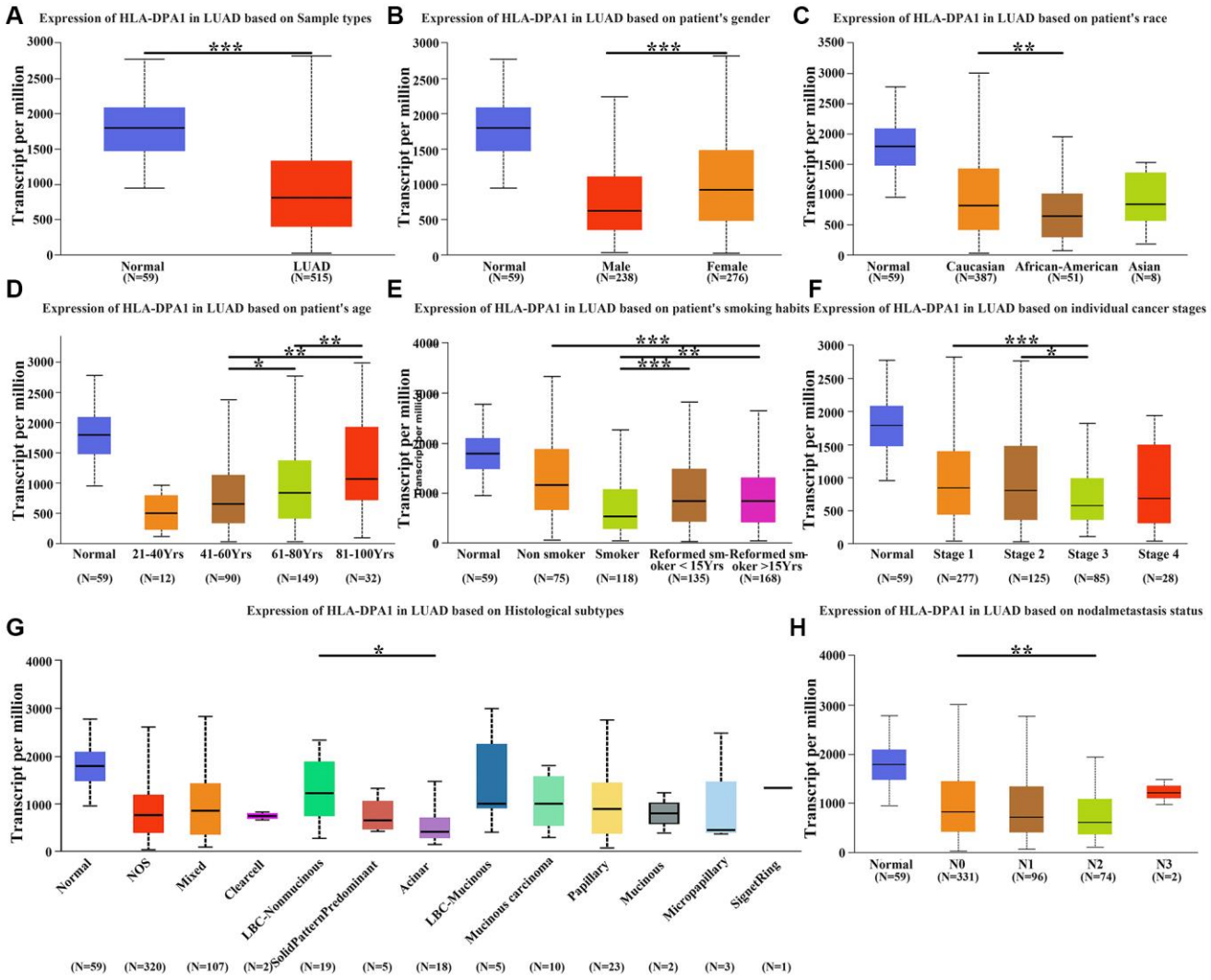

Figure 2. The correlation between the expression of HLA-DPA1 and demographic indicators in LUAD. (**A**) The expression of HLA-DPA1 in LUAD; its correlation with (**B**) Gender; (**C**) Race; (**D**) Age; (**E**) Smoking; (**F**) Clinical stage; (**G**) Tissue type; (**H**) Lymph node metastasis. Abbreviation: LUAD: lung adenocarcinoma; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

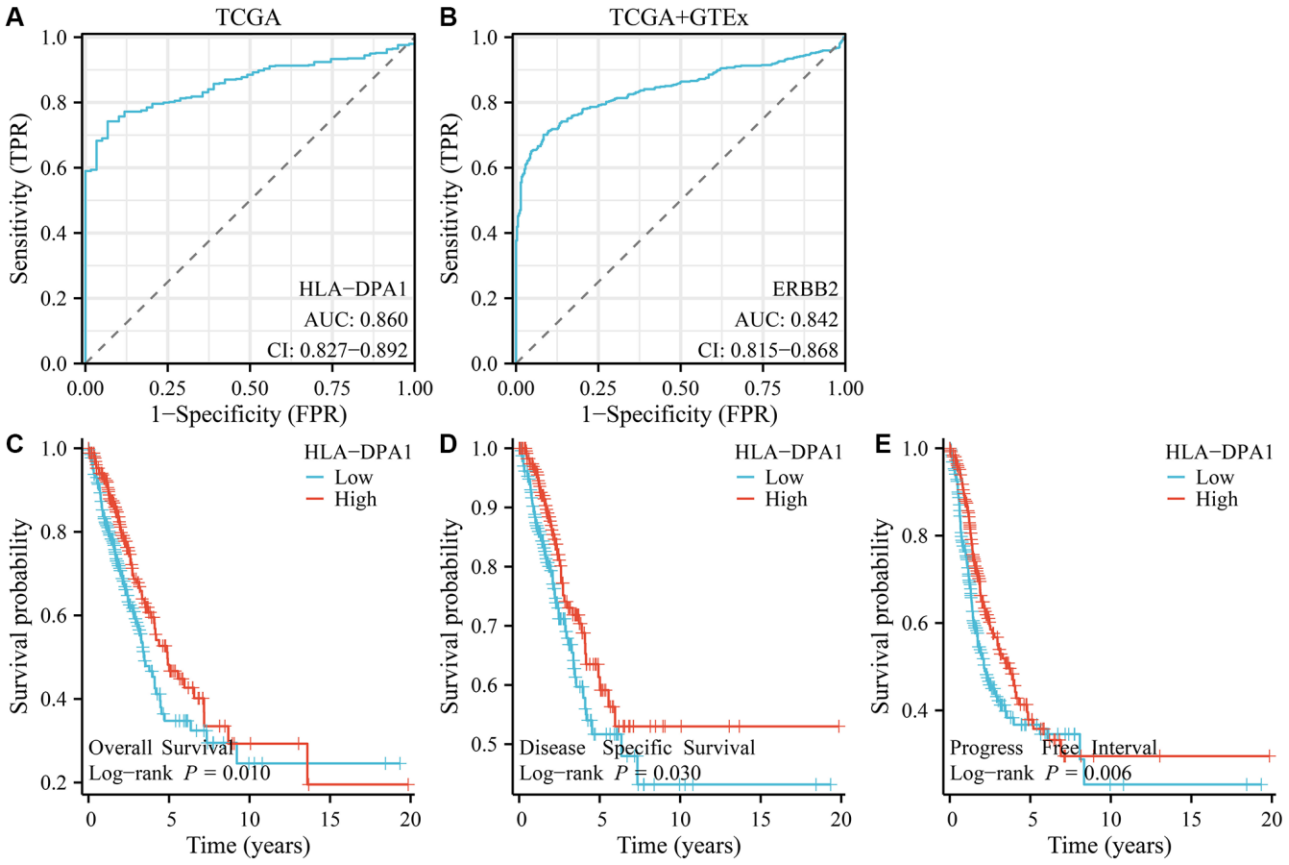

Figure 3. The correlation between the expression of HLA-DPA1 and the diagnosis and prognosis of patients with LUAD. (**A**, **B**) Diagnostic values of HLA-DPA1 in LUAD using ROC analysis; its correlation with (**C**) OS; (**D**) DSS; (**E**) PFI. Abbreviations: LUAD: lung adenocarcinoma; ROC: receiver operating characteristic; OS: overall survival; DSS: disease-specific survival; PFI: progress-free interval.

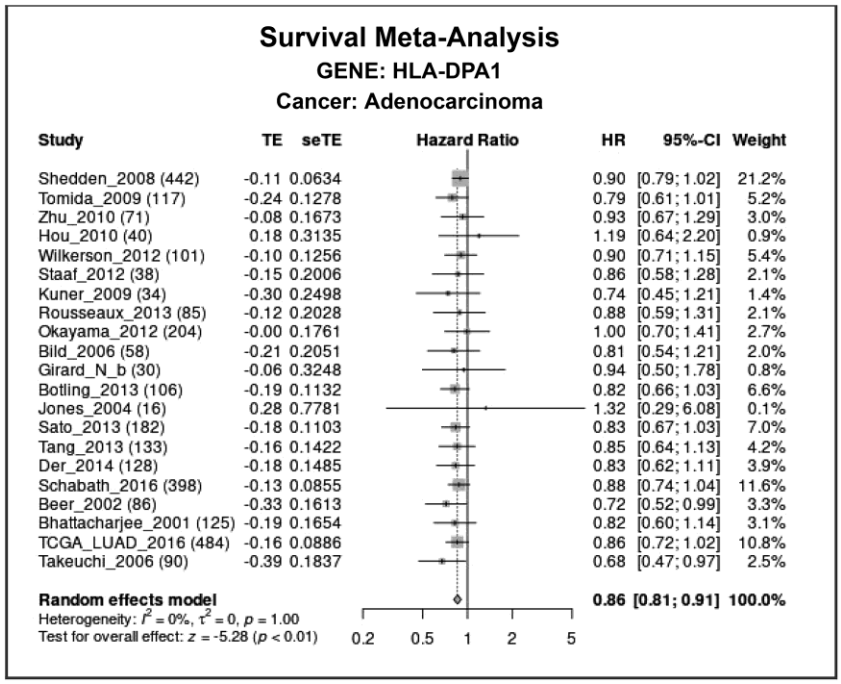

Figure 4. The association between a decreased expression of HLA-DPA1 and poor prognosis of patients with LUAD using the LCE database. Abbreviations: LUAD: lung adenocarcinoma; LCE: Lung Cancer Explorer.

HLA-DPA1 expression impacts cell proliferation, migration, and sensitivity to cisplatin in LUAD

HLA-DPA1 expression levels were significantly increased in LUAD cells compared to healthy cells, as confirmed by both Western blotting and polymerase chain reaction (PCR) (Figure 6A–6C). HLA-DPA1 overexpression resulted in a significant reduction in A549 and A549/DDP cell proliferation at both 48 h and 72 h, as demonstrated by CCK-8 assay (Figure 6D, 6E). Additionally, it increased the LUAD cell sensitivity to cisplatin, as depicted in Figure 6F. Finally, Transwell and wound healing assays revealed that HLA-DPA1 overexpression impacts the migration and invasion abilities of A549 and A549/DDP cells (Figures 7 and 8).

HLA-DPA1 expression and LUAD immune micro-environment

In LUAD tissues, decreased HLA-DPA1 expression was positively correlated with stromal scores (r = 0.495), immune scores (r = 0.706), and ESTIMATE scores (r = 0.655) (Figure 9A–9C). Stromal, immune, and ESTIMATE scores differed significantly between the high- and low-HLA-DPA1 expression groups (Figure 9D–9F). Additionally, we observed a negative correlation between HLA-DPA1 expression and multiple immune cell types, including cytotoxic cells (r = 0.506885853), regulatory T cells (Treg) (r = 0.452668941), mast cells (r = 0.452567257), eosinophils (r = 0.450615318), T follicular helper cells (TFH) (r = 0.43046312), neutrophils (r = 0.396488996), plasmacytoid dendritic cells (pDCs) (r = 0.366524994), B cells (r = 0.337705922), $CD8^+$ T cells (r = 0.321743937), T helper cells (r = 0.318979854), NK cells (r = 0.261549649), effector memory T cells (TEM) (r = 0.234554753), Th17 cells (r = 0.192560244), NK CD56dim cells (r = 0.19188812), central memory T cells (TCM) (r = 0.133514315), gamma delta T cells (TGD) (r = −0.144256151), and Th2 cells (r = −0.178080383) in LUAD (Figure 10 and Table 2). These differences in immune cell levels

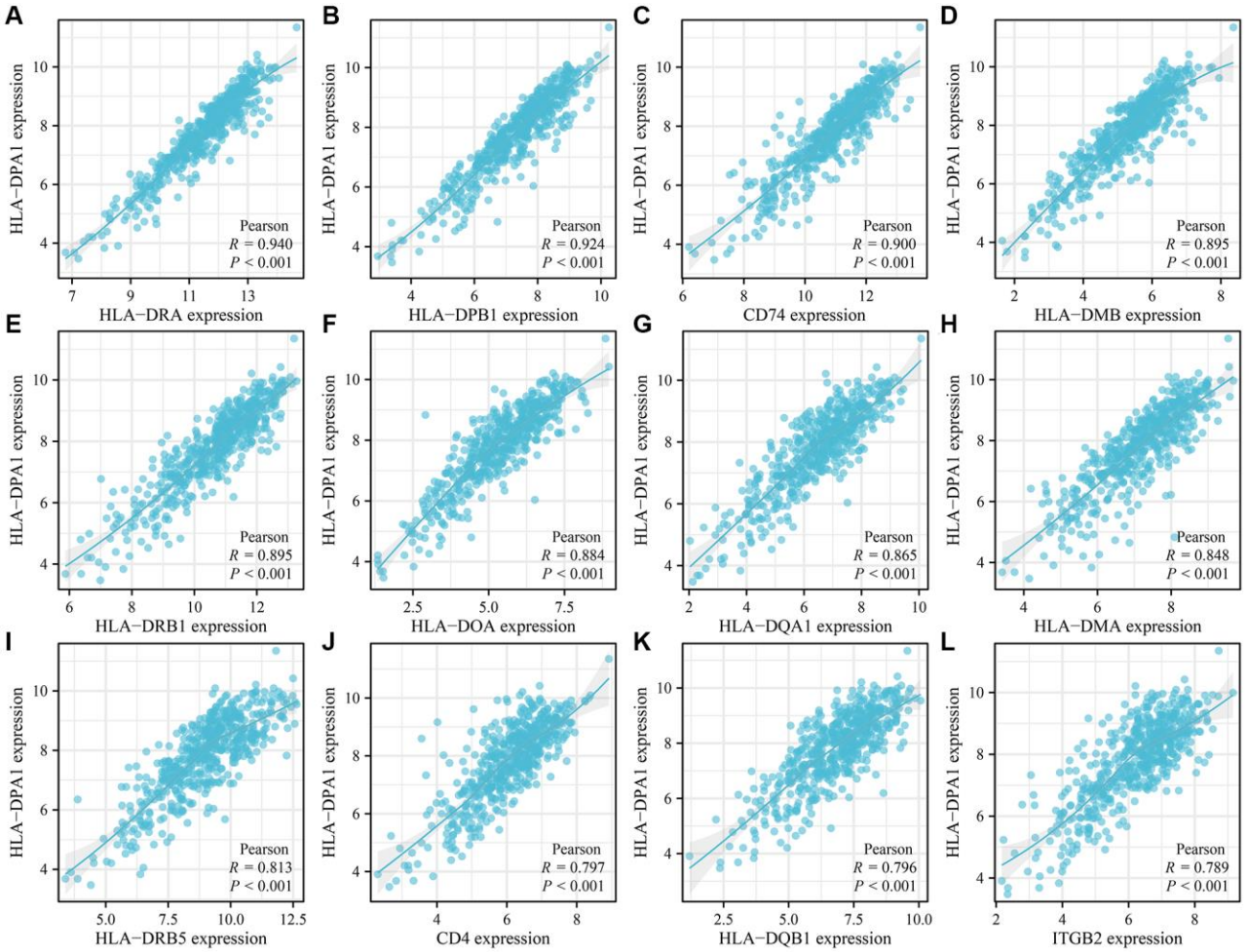

Figure 5. Genes positively co-expressed with HLA-DPA1 in LUAD. (**A**) HLA-DRA; (**B**) HLA-DPB1; (**C**) CD74; (**D**) HLA-DMB; (**E**) HLA-DRB1; (**F**) HLA-DOA; (**G**) HLA-DQA1; (**H**) HLA-DMA; (**I**) HLA-DRB5; (**J**) CD4; (**K**) HLA-DQB1; (**L**) ITGB2. Abbreviation: LUAD: lung adenocarcinoma.

Table 1. Pathways associated with HLA-DPA1 co-expressed genes using KEGG analysis in the DAVID database.

Term	Count	*P*
hsa04145: Phagosome	30	2.85E-24
hsa05150: Staphylococcus aureus infection	24	9.59E-22
hsa05140: Leishmaniasis	21	9.12E-20
hsa05416: Viral myocarditis	19	3.94E-19
hsa05330: Allograft rejection	16	3.39E-18
hsa04514: Cell adhesion molecules	25	8.44E-18
hsa05152: Tuberculosis	26	1.73E-17
hsa04640: Hematopoietic cell lineage	21	1.98E-17
hsa04612: Antigen processing and presentation	19	7.10E-17
hsa05332: Graft-versus-host disease	15	7.80E-16
hsa05320: Autoimmune thyroid disease	16	1.11E-15
hsa04940: Type I diabetes mellitus	15	1.14E-15
hsa05145: Toxoplasmosis	20	4.26E-15
hsa05310: Asthma	13	1.22E-14
hsa04672: Intestinal immune network for IgA production	14	2.57E-13
hsa05323: Rheumatoid arthritis	17	5.81E-13
hsa05322: Systemic lupus erythematosus	19	2.03E-12
hsa05166: Human T-cell leukemia virus 1 infection	21	1.47E-10
hsa05321: Inflammatory bowel disease	13	2.25E-10
hsa04613: Neutrophil extracellular trap formation	19	5.89E-10
hsa04380: Osteoclast differentiation	16	9.20E-10
hsa04658: Th1 and Th2 cell differentiation	14	1.25E-09
hsa05169: Epstein-Barr virus infection	19	1.60E-09
hsa04659: Th17 cell differentiation	14	9.35E-09
hsa05164: Influenza A	15	3.43E-07
hsa05133: Pertussis	9	2.06E-05
hsa04062: Chemokine signaling pathway	13	3.91E-05
hsa05170: Human immunodeficiency virus 1 infection	13	1.02E-04
hsa04666: Fc gamma R-mediated phagocytosis	9	1.20E-04
hsa04611: Platelet activation	10	1.22E-04
hsa04650: Natural killer cell mediated cytotoxicity	10	1.38E-04
hsa04670: Leukocyte transendothelial migration	9	3.69E-04
hsa04664: Fc epsilon RI signaling pathway	7	6.10E-04
hsa05417: Lipid and atherosclerosis	11	0.001790779
hsa04610: Complement and coagulation cascades	7	0.002090384
hsa05168: Herpes simplex virus 1 infection	18	0.002223244
hsa05340: Primary immunodeficiency	5	0.002689423
hsa05167: Kaposi sarcoma-associated herpesvirus infection	10	0.003079788
hsa05171: Coronavirus disease - COVID-19	11	0.003122867
hsa05221: Acute myeloid leukemia	6	0.003684199

hsa05135: Yersinia infection	8	0.005261833
hsa04060: Cytokine-cytokine receptor interaction	12	0.0058967
hsa05134: Legionellosis	5	0.011492318
hsa03250: Viral life cycle - HIV-1	5	0.016148607
hsa04620: Toll-like receptor signaling pathway	6	0.022359401
hsa05163: Human cytomegalovirus infection	9	0.02325965
hsa04662: B cell receptor signaling pathway	5	0.037971909
hsa05144: Malaria	4	0.041906079
hsa04015: Rap1 signaling pathway	8	0.044169696

Abbreviation: KEGG, Kyoto Encyclopedia of Genes and Genomes.

between HLA-DPA1 high-expression and low-expression groups are illustrated in Supplementary Figure 1. Using the Tumor Immune Estimation Resource (TIMER) database, we found a similar trend that a decreased HLA-DPA1 expression in LUAD was associated with reductions in immune cell populations, including B cells (r = 0.547), $CD8^+$ T cells (r = 0.444), $CD4^+$ T cells (r = 0.437), macrophages (r = 0.448), dendritic cells (r = 0.783), and neutrophils (r = 0.516) (Figure 11). Additionally, a significant inverse correlation was observed between HLA-DPA1 expression and tumor purity (r = −0.366).

HLA-DPA1 expression was correlated with immune cell markers in LUAD

Using data from the TIMER database, we revealed a significant correlation between the decreased expression of HLA-DPA1 and T cell exhaustion as well as markers of various immune cell types, including $CD8^+$ T cells, T cells, B cells, monocytes, tumor-associated macrophages, macrophages, neutrophils, natural killer cells, dendritic cells, T helper cells, and Treg (Figure 12 and Table 3). Furthermore, in the context of tumor purity, correlation trends remained consistent in LUAD

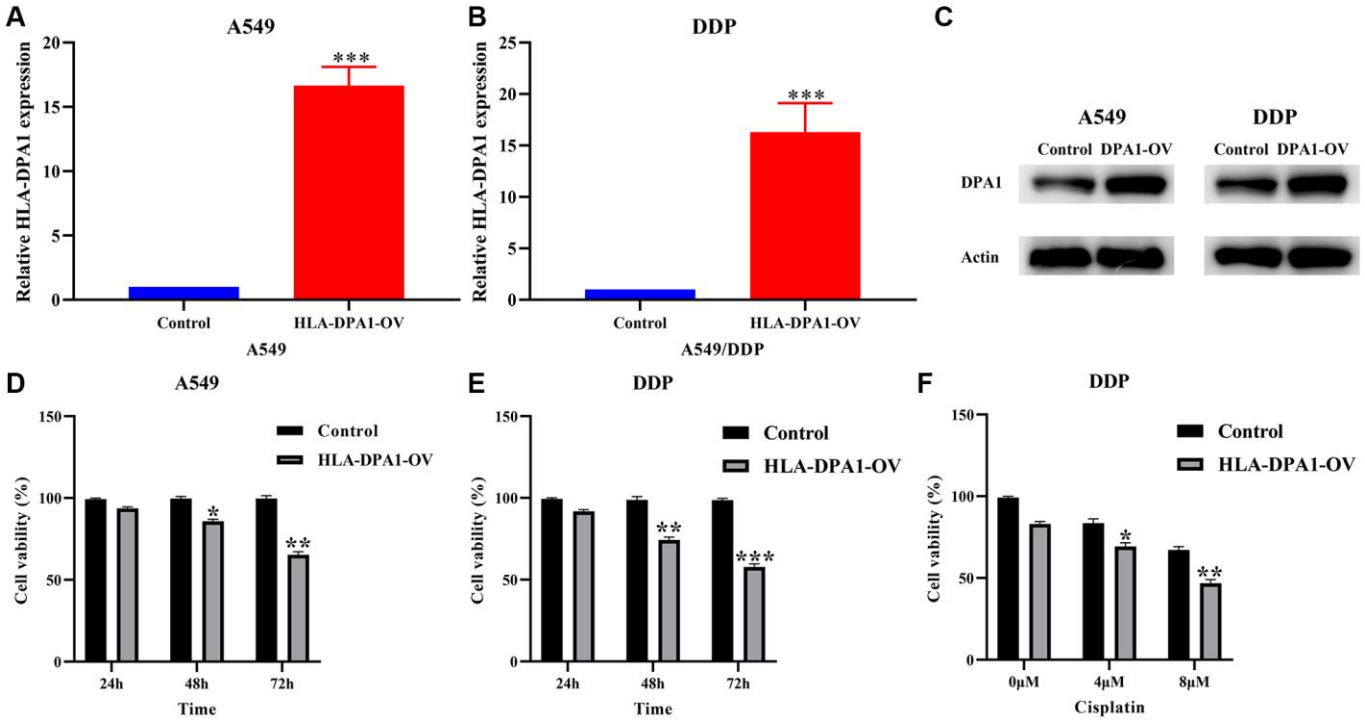

Figure 6. HLA-DPA1 overexpression inhibits cancer cell proliferation and promotes cell sensitivity to cisplatin in LUAD. (**A**–**C**) A549 and A549/DDP cell assays using RT-PCR and Western blotting (**D**, **E**) Assessment of A549 and A549/DDP cell proliferation; (**F**) A549/DDP cell sensitivity to cisplatin. Abbreviation: LUAD: lung adenocarcinoma.

(Table 4 and Supplementary Figure 2). We found similar correlations using Gene Expression Profiling Interactive Analysis (GEPIA) (Table 5 and Figure 13). Genes significantly associated with decreased HLA-DPA1 expression in LUAD tissues included those encoding various immune cell markers, such as CD8A, CD8B, CD3D, CD2, CD3E, CD19, CD79A, CD86, CSF1R, CCL2, CD68, IL10, IRF5, PTGS2, CD163, VSIG4, MS4A4A, CEACAM8, ITGAM, CCR7, IL21, STAT3, HLA-DPB1, HLA-DQB1, HLA-DRA, CD1C, NRP1, ITGAX, TBX21, TNF, STAT4, STAT1, IFNG, STAT6, STAT5A, IL13, FOXP3, CCR8, STAT5B, TGFB1, PDCD1, CTLA4, LAG3, and HAVCR2.

DISCUSSION

In recent years, LUAD emerged as a prevalent subtype of non-small-cell lung cancer due to its increasing incidence [9–11]. Genetic alterations substantially impact the progression of lung cancer and can be indicative of poor prognosis in patients with cancer. Notably, GALNT2 overexpression in LUAD tissues is associated with worse patient outcomes. *In vitro* experiments demonstrate that downregulation of GALNT2 suppresses cell proliferation, migration, and invasion, while its over-expression accelerates these processes and activates

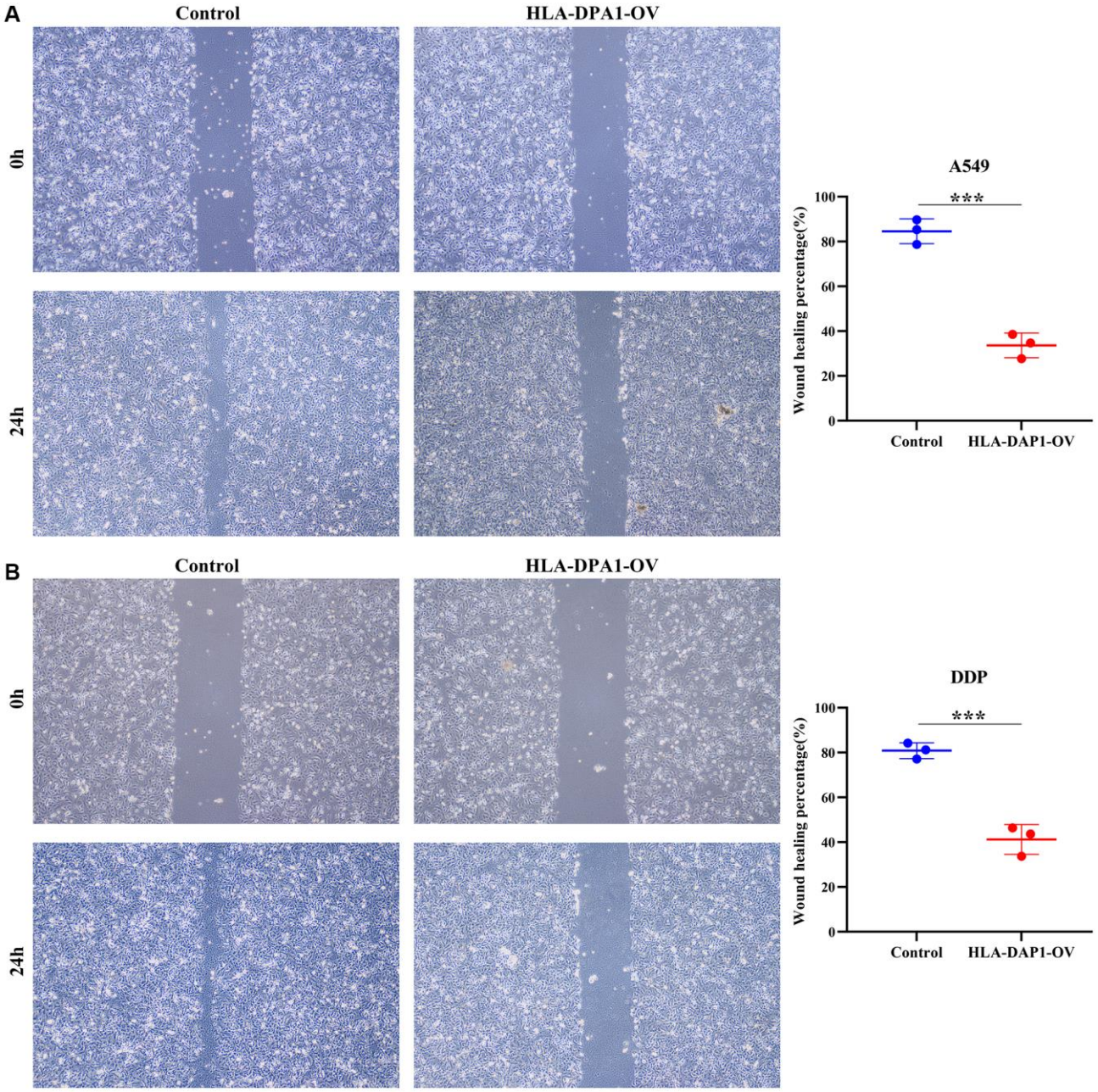

Figure 7. HLA-DPA1 overexpression inhibits LUAD cell migration. (**A**) A549; (**B**) A549/DDP. Abbreviation: LUAD: lung adenocarcinoma.

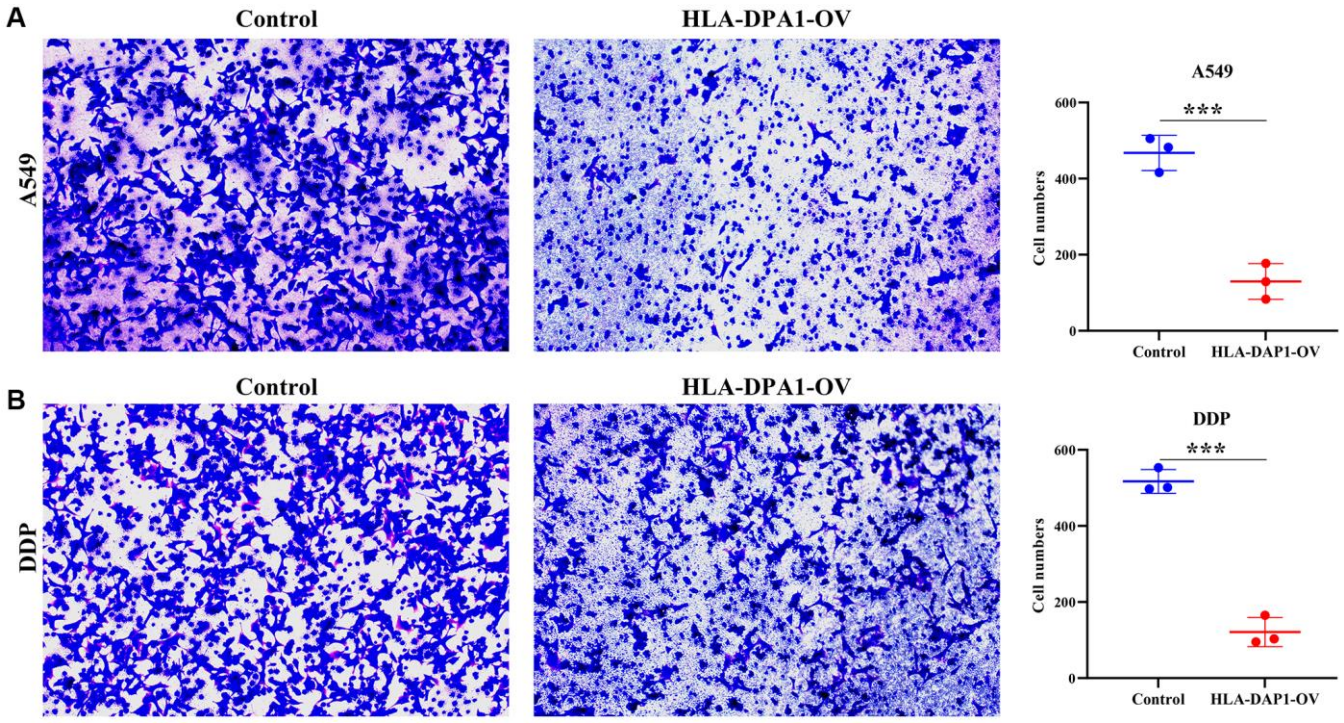

Figure 8. HLA-DPA1 overexpression inhibits LUAD cell invasion. (**A**) A549; (**B**) A549/DDP. Abbreviation: LUAD: lung adenocarcinoma.

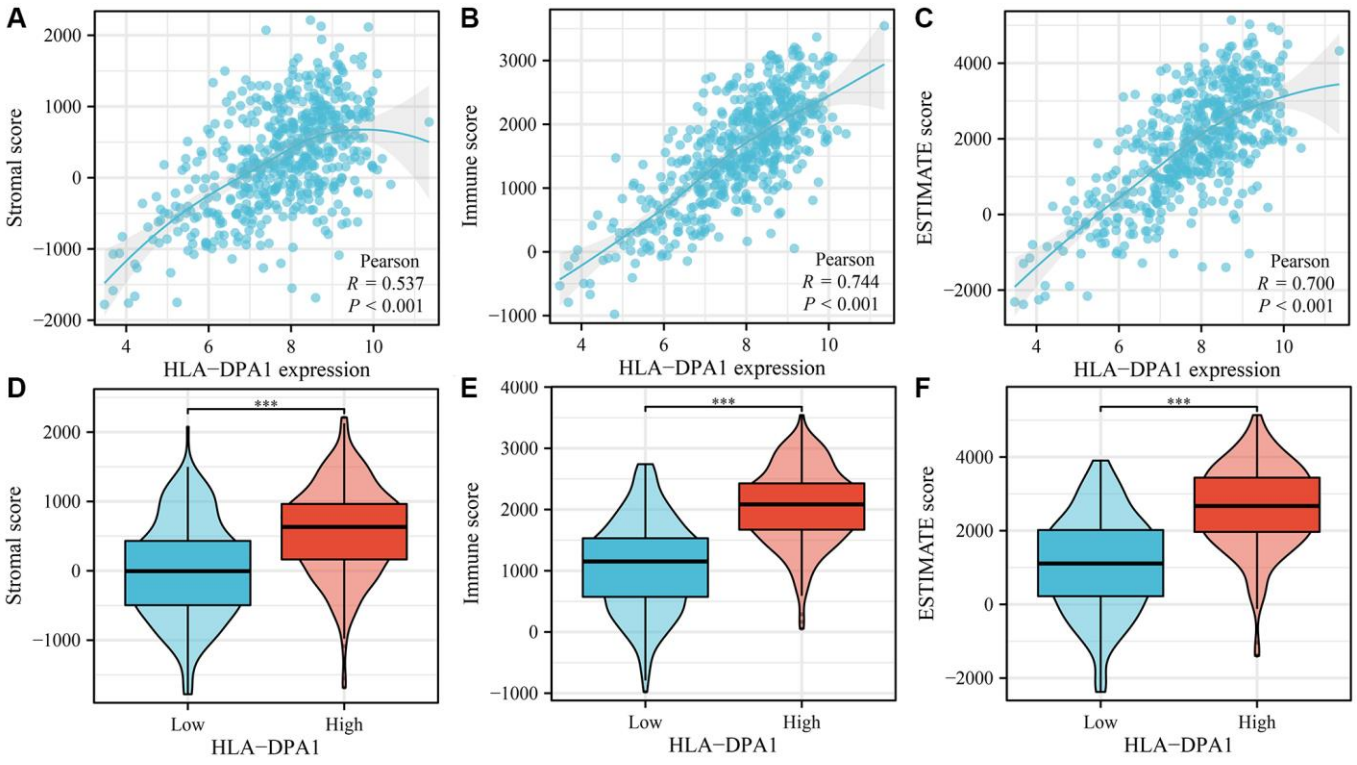

Figure 9. The correlation between the HLA-DPA1 overexpression and stromal, immune, and ESTIMATE scores in LUAD. (**A**–**C**) Correlation analysis with stromal, immune, and ESTIMATE scores; (**D**–**F**) Stromal, immune, and ESTIMATE scores in high- and low-HLA-DPA1 expression groups. Abbreviation: LUAD: lung adenocarcinoma.

Notch/Hairy and Enhancer of Split 1/phosphatase and tensin homolog/phosphatidylinositol 3-kinase/protein kinase B signaling, inducing cancerous transformation [9]. Similarly, MRPL42 overexpression is observed in the early stages of LUAD. Knockdown of MRPL42 expression reduces cell proliferation and colony formation, induces cell cycle arrest, and inhibits migration and invasion of LUAD cells [10]. However, the relationship between HLA-DPA1 and LUAD remains unexplored. Our analysis revealed decreased HLA-DPA1 expression in LUAD tissues, associated with gender, race, age, smoking history, clinical stage, histological type, and lymph node metastasis of patients with LUAD. Furthermore, HLA-DPA1 expression has diagnostic value and is correlated with OS, DSS, and PFI of patients with LUAD. Our preliminary findings suggest that HLA-DPA1 may serve as a potential prognostic marker in this pathology.

DCs are vital for antigen presentation and cancer progression. They process foreign antigens and present them to $CD4^+$ T cells, with activated DCs changing MHC class II antigen presentation mechanisms to $CD4^+$ T cells [12]. PD-L1, expressed on tumor-related DCs in patients with lung cancer, binds to the B7.1 receptor. Blocking PD-L1 on DCs reduces its binding to B7.1, enhancing T cell activation and increasing OS in patients with non-small cell lung cancer receiving PD-L1 blocker therapy [13]. HLA-DPA1 is a marker of DCs, and HLA-DPA1 co-expressed genes are involved in various biological processes such as inflammatory response, positive regulation of T cell activation,

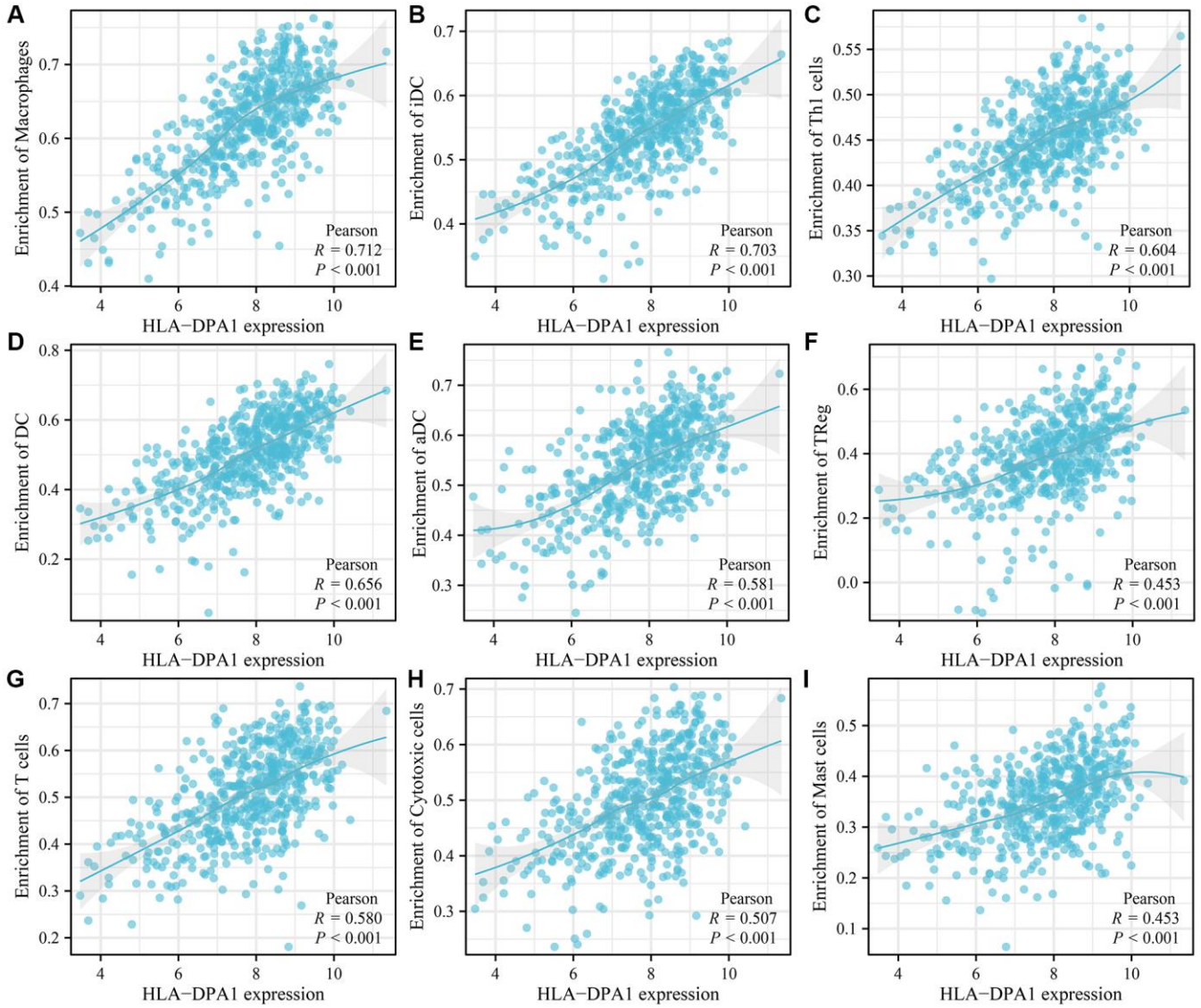

Figure 10. The correlation between the HLA-DPA1 overexpression and immune cell levels in LUAD using data from The Cancer Genome Atlas. (**A**) Macrophages; (**B**) iDC; (**C**) Th1 cells; (**D**) DC; (**E**) aDC; (**F**) TReg; (**G**) T cells; (**H**) Cytotoxic cells; (**I**) Mast cells. Abbreviations: LUAD: lung adenocarcinoma; DC: dendritic cells; Treg: regulatory T cells.

Table 2. The association between the HLA-DPA1 overexpression and immune cells in LUAD.

Gene	Immune cells	Coefficient	*P*
HLA-DPA1	Macrophages	0.71192265	1.78742E-84
HLA-DPA1	iDC	0.702589054	2.18051E-81
HLA-DPA1	DC	0.655642609	1.57971E-67
HLA-DPA1	Th1 cells	0.603802207	7.53079E-55
HLA-DPA1	aDC	0.580758414	6.28424E-50
HLA-DPA1	T cells	0.580356886	7.59546E-50
HLA-DPA1	Cytotoxic cells	0.506885853	1.58675E-36
HLA-DPA1	TReg	0.452668941	1.37973E-28
HLA-DPA1	Mast cells	0.452567257	1.4236E-28
HLA-DPA1	Eosinophils	0.450615318	2.59103E-28
HLA-DPA1	TFH	0.43046312	1.00564E-25
HLA-DPA1	Neutrophils	0.396488996	9.79151E-22
HLA-DPA1	pDC	0.366524994	1.39729E-18
HLA-DPA1	B cells	0.337705922	7.62353E-16
HLA-DPA1	CD8 T cells	0.321743937	1.90451E-14
HLA-DPA1	T helper cells	0.318979854	3.26241E-14
HLA-DPA1	NK cells	0.261549649	7.01338E-10
HLA-DPA1	Tem	0.234554753	3.59003E-08
HLA-DPA1	Th17 cells	0.192560244	6.71982E-06
HLA-DPA1	NK CD56dim cells	0.19188812	7.245E-06
HLA-DPA1	Tcm	0.133514315	0.001893543
HLA-DPA1	NK CD56bright cells	0.011507791	0.789809688
HLA-DPA1	Tgd	−0.144256151	0.000782494
HLA-DPA1	Th2 cells	−0.178080383	3.20957E-05

Abbreviation: LUAD: lung adenocarcinoma.

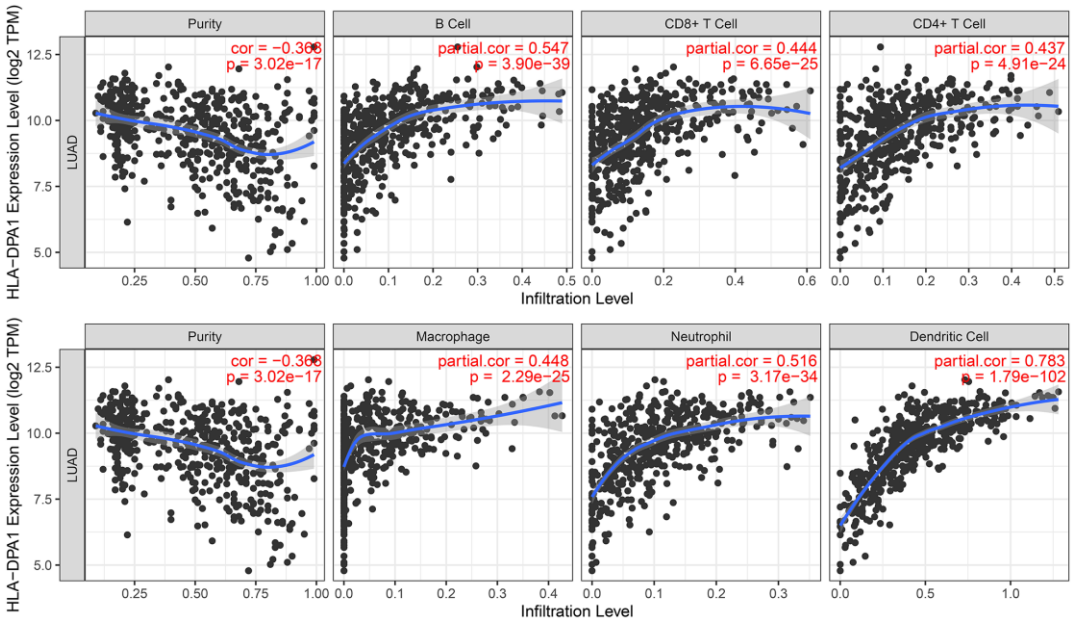

Figure 11. The association between the HLA-DPA1 overexpression and tumor purity and immune cells in LUAD using the TIMER database. Abbreviations: LUAD: lung adenocarcinoma; TIMER: Time Interval Medical Event Recorder.

antigen processing and presentation, adaptive immune response, and T cell-mediated cytotoxicity, among others. Our study further revealed that HLA-DPA1 overexpression could inhibit cell proliferation and migration, as well as promote cell sensitivity to cisplatin. These findings indicate that HLA-DPA1 is involved in LUAD progression as a tumor suppressor gene.

The relationship between immune cells, immune-related factors, and cancer progression has been well-established [14–18]. For instance, FAM83H overexpression inhibits infiltration of tumor-infiltrating lymphocytes (TILs) and anti-tumor activity, particularly that of $CD8^+$ T cells. Its overexpression level inversely correlates with the expression levels of CD8A, CD8B, CD2, CD3D, and CD3E [14]. In colorectal cancer, increased sCD163 expression indicates poorer OS and DFS, with high sCD163 levels and monocytes significantly influencing DFS in these patients [16]. Our study establishes a link

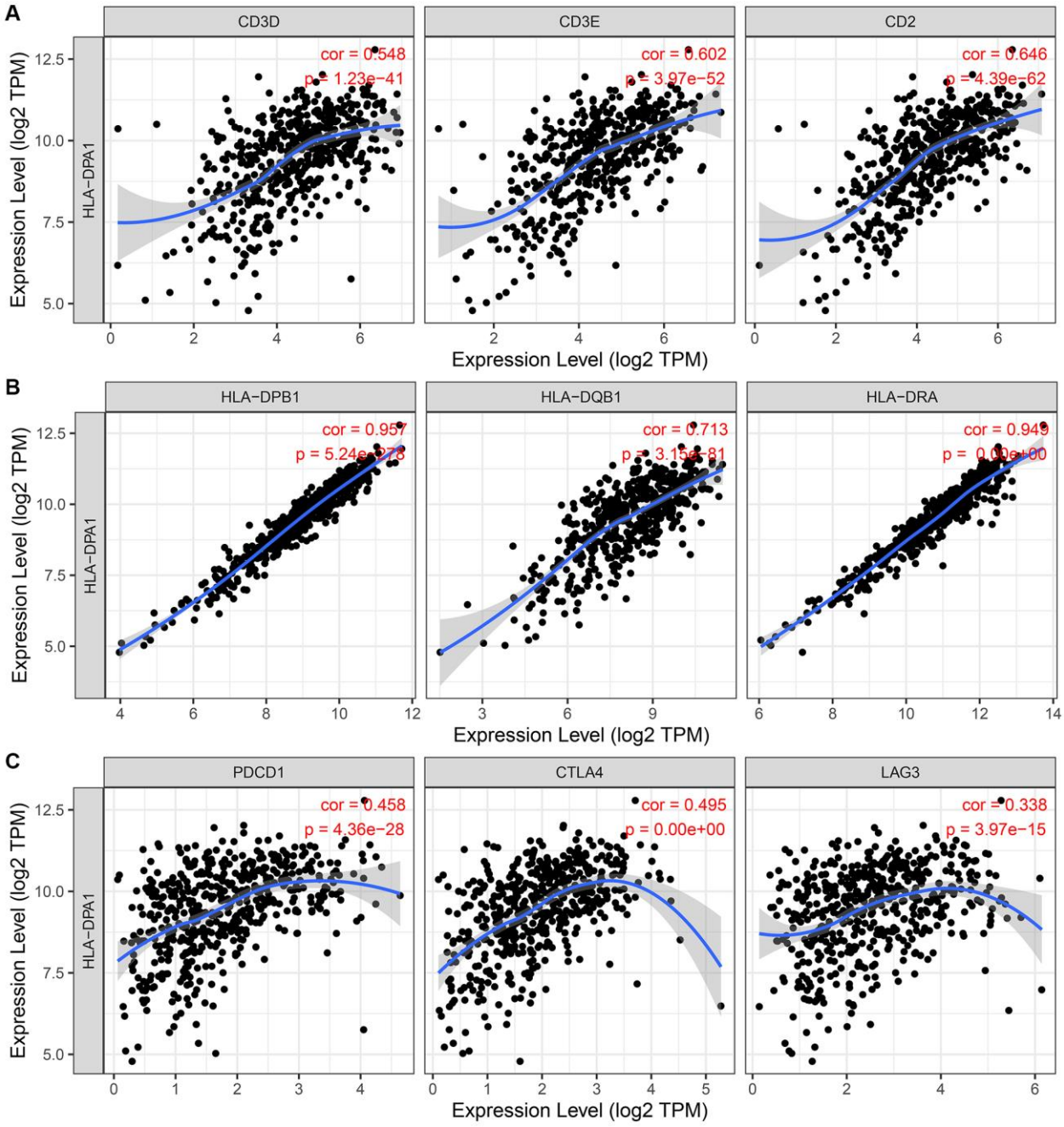

Figure 12. The association between the HLA-DPA1 overexpression and immune cell markers in LUAD using the TIMER database. (**A**) T cell markers; (**B**) Dendritic cell markers; (**C**) T cell exhaustion markers. Abbreviations: DC: dendritic cells; LUAD: lung adenocarcinoma.

Table 3. The correlation between the HLA-DPA1 expression and the level of immune cell markers in LUAD.

Gene	Coefficient	*P*	Gene	Coefficient	*P*
CD8A	0.463860846	***	HLA-DPB1	0.957048805	***
CD8B	0.383594772	***	HLA-DQB1	0.713309359	***
CD3D	0.547637137	***	HLA-DRA	0.948978566	***
CD3E	0.602153884	***	CD1C	0.622583525	***
CD2	0.645701555	***	NRP1	0.260608494	***
CD19	0.328131252	***	ITGAX	0.590846161	***
CD79A	0.301133316	***	TBX21	0.497153269	***
CD86	0.717281395	***	STAT4	0.56603653	***
CSF1R	0.720382056	***	STAT1	0.349391568	***
CCL2	0.40749069	***	IFNG	0.313908839	***
CD68	0.601204653	***	TNF	0.43121415	***
IL10	0.528217991	***	GATA3	0.448382268	***
NOS2	0.024972061	0.572	STAT6	0.240980432	***
IRF5	0.48368983	***	STAT5A	0.627124519	***
PTGS2	−0.149620779	***	IL13	0.191836762	***
CD163	0.551646778	***	BCL6	0.093426945	*
VSIG4	0.615448587	***	IL21	0.234191463	***
MS4A4A	0.647014328	***	STAT3	0.115660054	**
CEACAM8	0.387397902	***	IL17A	0.156174024	***
ITGAM	0.693646404	***	FOXP3	0.557837581	***
CCR7	0.573222298	***	CCR8	0.564637826	***
KIR2DL1	0.080908061	0.067	STAT5B	0.255361072	***
KIR2DL3	0.141185802	**	TGFB1	0.47796272	***
KIR2DL4	0.097084787	*	PDCD1	0.458139335	***
KIR3DL1	0.109261553	*	CTLA4	0.4946446	***
KIR3DL2	0.195677599	***	LAG3	0.338210486	***
KIR3DL3	−0.018825523	0.670	HAVCR2	0.72004897	***
KIR2DS4	0.14515847	***	GZMB	0.193203672	***

Abbreviation: LUAD: lung adenocarcinoma; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Table 4. The correlation between the HLA-DPA1 overexpression and immune cell markers in the context of tumor purity.

Gene	Coefficient	*P*	Gene	Coefficient	*P*
CD8A	0.361547657	***	HLA-DPB1	0.951444602	***
CD8B	0.295531118	***	HLA-DQB1	0.678456948	***
CD3D	0.44980375	***	HLA-DRA	0.943553019	***
CD3E	0.516992518	***	CD1C	0.589149871	***
CD2	0.57140071	***	NRP1	0.240701613	***
CD19	0.191985178	***	ITGAX	0.517238257	***
CD79A	0.163961649	***	TBX21	0.407921541	***
CD86	0.664542948	***	STAT4	0.493977846	***
CSF1R	0.669761973	***	STAT1	0.266515617	***
CCL2	0.324125987	***	IFNG	0.213157882	***
CD68	0.542372297	***	TNF	0.335546484	***

IL10	0.444583773	***	GATA3	0.348966526	***
NOS2	-0.057981416	0.199	STAT6	0.275594139	***
IRF5	0.425447924	***	STAT5A	0.560979818	***
PTGS2	-0.163392491	***	IL13	0.123975663	**
CD163	0.478418571	***	BCL6	0.082693089	0.067
VSIG4	0.566379418	***	IL21	0.161538758	***
MS4A4A	0.588284315	***	STAT3	0.127863377	**
CEACAM8	0.39364805	***	IL17A	0.08745736	0.052
ITGAM	0.647768352	***	FOXP3	0.474996557	***
CCR7	0.485288268	***	CCR8	0.487678859	***
KIR2DL1	0.017664227	0.696	STAT5B	0.24347394	***
KIR2DL3	0.062591704	0.165	TGFB1	0.417646704	***
KIR2DL4	0.011009074	0.807	PDCD1	0.356089872	***
KIR3DL1	0.042372557	0.348	CTLA4	0.384848895	***
KIR3DL2	0.115349466	*	LAG3	0.238139944	***
KIR3DL3	-0.05426989	0.229	HAVCR2	0.668044203	***
KIR2DS4	0.076415879	0.090	GZMB	0.058566398	0.194

Abbreviation: LUAD: lung adenocarcinoma; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Table 5. The association between the HLA-DPA1 overexpression and immune cell markers in LUAD tissues from the GEPIA database.

Gene	Coefficient	*P*	Gene	Coefficient	*P*
CD8A	0.35	***	CD1C	0.42	***
CD8B	0.23	***	NRP1	0.27	***
CD3D	0.39	***	ITGAX	0.40	***
CD3E	0.49	***	TBX21	0.11	*
CD2	0.54	***	STAT4	0.41	***
CD19	0.24	***	STAT1	0.29	***
CD79A	0.20	***	IFNG	0.24	***
CD86	0.62	***	TNF	0.3	***
CSF1R	0.59	***	GATA3	0.0024	0.96
CCL2	0.23	***	STAT6	0.27	***
CD68	0.49	***	STAT5A	0.56	***
IL10	0.44	***	IL13	0.21	***
IRF5	0.32	***	BCL6	0.14	0.068
PTGS2	-0.14	**	IL21	0.29	***
CD163	0.32	***	STAT3	0.17	***
VSIG4	0.46	***	IL17A	0.12	0.072
MS4A4A	0.49	***	FOXP3	0.48	***
CEACAM8	0.25	***	CCR8	0.48	***
ITGAM	0.54	***	STAT5B	0.25	***
CCR7	0.43	***	TGFB1	0.32	***
KIR3DL2	-0.061	0.18	PDCD1	0.30	***
HLA-DPB1	0.91	***	CTLA4	0.30	***
HLA-DQB1	0.48	***	LAG3	0.14	**
HLA-DRA	0.88	***	HAVCR2	0.59	***

Abbreviation: LUAD: lung adenocarcinoma; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

between the decreased expression of HLA-DPA1 and various immune cell subsets, such as Treg, mast cells, eosinophils, TFH, neutrophils, pDCs, B cells, $CD8^+$ T cells, T helper cells, NK cells, Tem, Th17 cells, NK CD56dim cells, Tcm, Tgd, and Th2 cells. Additionally, we identified associations with the markers of overall immune response, such as CD8A and CD8B for $CD8^+$ T cells, CD3D, CD3E, and CD2 for T cells, and CD19 and CD79A for B cells, as well as those of T cell exhaustion such as PDCD1, CTLA4, lymphocyte activation gene 3, hepatitis A virus cellular receptor 2, and granzyme B. Our findings were consistent in LUAD tissues, as corroborated using GEPIA. This highlights the fundamental role of HLA-DPA1 in the LUAD immune microenvironment.

Our study employed robust methodologies by combining data from TCGA and GEO databases for comprehensive bioinformatics analysis and conducting cell experiments to explore the role of HLA-DPA1 in LUAD progression. The incorporation of large datasets and cell experiments enhanced the reliability and validity of our findings. However, further research is needed to elucidate the mechanisms of HLA-DPA1 in LUAD progression and the underlying signaling pathways. Through our research, we revealed that

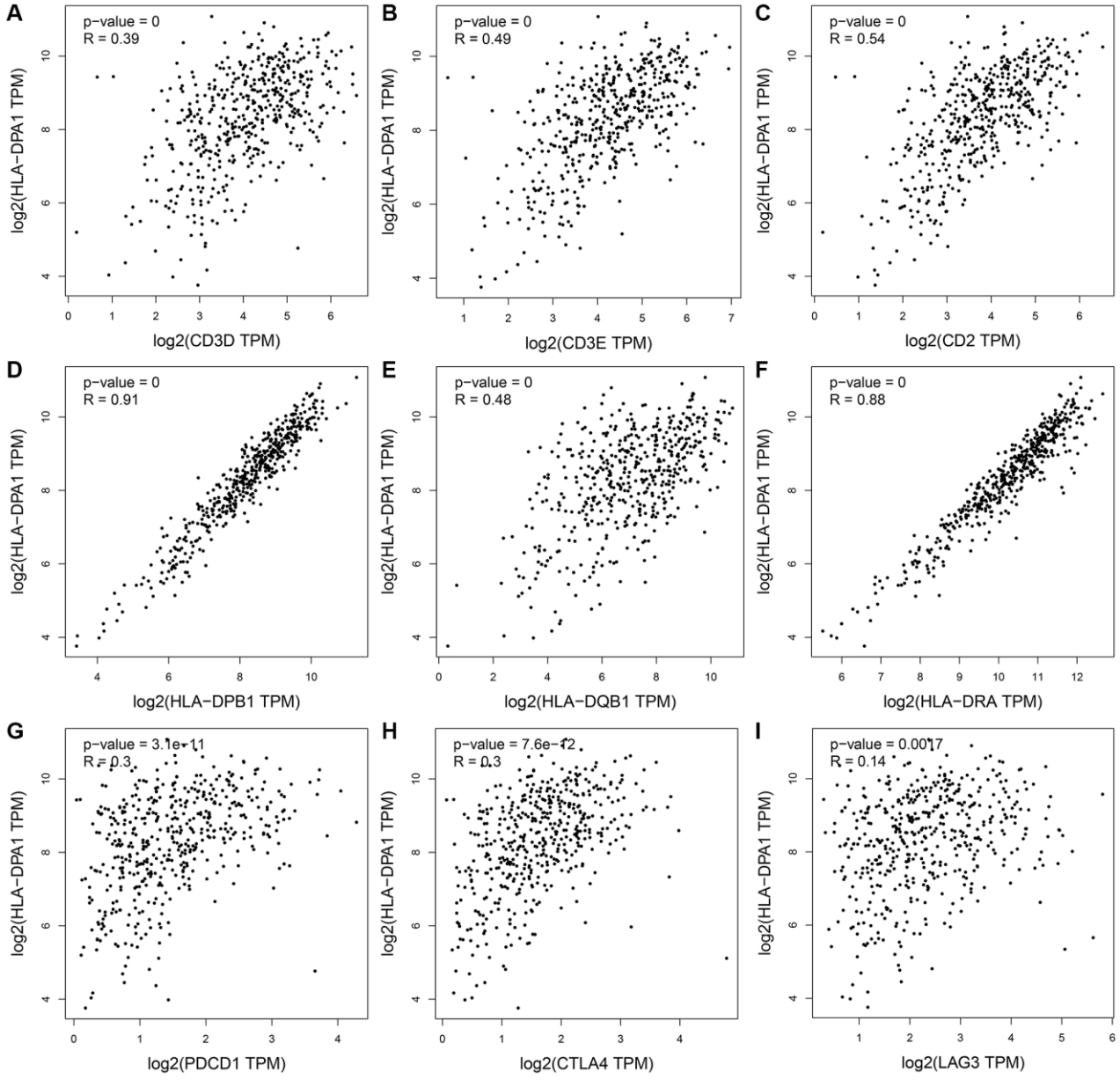

Figure 13. The association between the HLA-DPA1 overexpression and immune cell markers in LUAD using the GEPIA database. (**A**) CD3D; (**B**) CD3E; (**C**) CD2; (**D**) HLA-DPB1; (**E**) HLA-DQB1; (**F**) HLA-DRA; (**G**) PDCD1; (**H**) CTLA4; (**I**) LAG3. Abbreviation: LUAD: lung adenocarcinoma.

HLA-DPA1 expression was significantly decreased in LUAD tissues and correlated with various clinical parameters. Furthermore, lower HLA-DPA1 expression was associated with reduced LUAD immune scores, while HLA-DPA1 overexpression was shown to inhibit cell proliferation and migration while promoting cell sensitivity to cisplatin.

CONCLUSIONS

Decreased HLA-DPA1 expression is associated with poor prognosis, immune infiltration, cancer cell proliferation, and progression in LUAD. Our findings highlight the potential of HLA-DPA1 as both a prognostic biomarker and a therapeutic target for LUAD.

MATERIALS AND METHODS

Retrieval of data from TCGA and XENA databases

We downloaded expression data for 59 normal tissues and 535 samples of LUAD tissues from the TCGA (https://portal.gdc.cancer.gov/) database. Gene expression levels were measured in transcripts per million (TPM). Additionally, we downloaded data for 288 normal tissues from the GTEx database in XENA (https://xena.ucsc.edu/). Using TCGA database, we obtained clinical data from patients with LUAD, including cancer patient survival data updated up to January 2023.

Retrieval of data from the GEO database

mRNA data from both LUAD and normal lung tissues were obtained from four relevant data sets, including GSE68571, GSE32863, GSE10072, and GSE2514 from the GEO (https://www.ncbi.nlm.nih.gov/geo) database. We retrieved series_matrix and platform annotation information from these datasets to verify the expression levels of HLA-DPA1 in LUAD based on gene annotation.

Retrieval of data from the GEPIA database

To conduct further analyses, we utilized the GEPIA (http://gepia.cancer-pku.cn) database to analyze gene expression in relation to prognosis and other genes within LUAD [19]. We utilized the Pearson method within the correlation module of the GEPIA database to validate the relationship between HLA-DPA1 expression and immune cell markers in LUAD tissues.

Retrieval of data from the UALCAN database

We utilized the UALCAN (http://ualcan.path.uab.edu) database to evaluate the HLA-DPA1 expression in both LUAD and normal tissues, focusing on its correlation with key clinical characteristics, such as cancer stage, tumor grade, race, and gender. Our study relied on data from the TCGA database, available within the UALCAN database [20].

Retrieval of data from the TIMER database

The TIMER database is a widely employed tool in cancer research for investigating immune infiltrating cells [21]. We utilized the gene module within the TIMER (https://cistrome.shinyapps.io/timer/) database to analyze the relationship between HLA-DPA1 expression and immune infiltrating cells. Furthermore, we examined correlations between HLA-DPA1 expression and immune cell biomarkers specific to LUAD using the correlation analysis module.

Diagnostic and prognostic values of HLA-DPA1

The diagnostic value of HLA-DPA1 in normal and LUAD tissues was assessed using receiver operating characteristic (ROC) analysis, with the area under the curve serving as the evaluation metric. Patient data on HLA-DPA1 expression were grouped by the median value, and survival analysis was employed to explore the relationship between decreased HLA-DPA1 expression and prognostic indicators in patients with LUAD. To evaluate the impact of decreased HLA-DPA1 expression on OS in patients with LUAD, we conducted a meta-analysis using the LCE (https://lce.biohpc.swmed.edu/) database.

HLA-DPA1 co-expressed genes

In this study, we employed Pearson correlation analysis to identify genes strongly associated with HLA-DAP1 expression in LUAD tissues. A correlation was considered significant when the correlation coefficient (r) was greater than 0.6 or less than −0.6.

Biological functions, and pathways of HLA-DPA1

Using the DAVID (https://david.ncifcrf.gov/) and TISIDB (http://cis.hku.hk/TISIDB/) databases, we performed GO and KEGG analyses to identify the pathways associated with the previously identified individual or multiple genes [22]. We used the DAVID database to identify significant functional annotations and mechanisms associated with genes that co-express with HLA-DPA1, applying a screening criterion based on *P*-values < 0.05. In addition to the DAVID analysis, we inputted the gene HLA-DPA1 into the TISIDB database to specifically investigate its functions and mechanisms.

Cell models of HLA-DPA1 overexpression

A549 and A549/DDP cells were cultured in RPMI-1640 medium with 10% fetal bovine serum (FBS). The A549/DDP cells were cultured with 1 μg/mL cisplatin. HLA-DPA1 expression was induced by transfection using lipofectamine 3000 (Thermo Fisher Scientific, USA) according to the instructions of the manufacturer. The cells were cultured in optimal cell growth conditions in 6-well plates. The resulting expression was verified using reverse transcription-polymerase chain reaction (RT-PCR) and Western blotting. The NCBI identification number for HLA-DPA1 was NM_001242525.

RT-PCR

Following cell transfection, RNA was extracted from A549 and A549/DDP cells using an RNA lysis solution. After quantification and normalization of RNA, reverse transcription was carried out to synthesize cDNA. The relative expression of HLA-DPA1 in both A549 and A549/DDP cells was calculated by subjecting the cDNA to PCR amplification using primers obtained from GeneCopoeia (China). The primer identification numbers provided for actin and HLA-DPA1 were HQP108762 and HQP008859, respectively.

Western blotting

Total protein from A549 and A549/DDP cells was extracted using protein lysis solution following transfection. The BCA Kit (Solarbio, China) was employed to quantify and normalize protein levels. Western blotting was performed using standard methodologies for primary and secondary antibody incubations. The concentrations of tubulin (No. 11224-1-AP, Proteintech, China) and HLA-DPA1 (No. 16109-1-AP, Proteintech, China) were 1:5000 and 1:2000, respectively. The relative HLA-DPA1 expression levels in A549 and A549/DDP cells were determined after conducting the Western blotting.

CCK-8 assay

Following the successful transfection of A549 and A549/DDP cells, viable cells were counted and plated at a density of 3,000 cells/well in a 96-well plate. Once the attachment was confirmed, cell activity was measured in both the control and HLA-DPA1 overexpression groups. The impact of cisplatin on cancer cell viability was assessed at concentrations of 0 μm, 4 μm, and 8 μm.

Wound healing assay

After transfection, A549 and A549/DDP cells were counted and plated in 6-well plates. Straight lines were drawn on the surface of the plates using a 200 μl pipette tip. We included control and HLA-DPA1 overexpression groups. Photographs were taken after rinsing the wells with phosphate buffer solution (PBS) at 24 and 48 hours, respectively. Cell migration in the control and HLA-DPA1 overexpression groups was assessed, and results were compared for statistical significance.

Transwell assay

The Matrigel was diluted and used to coat the bottom of the Transwell chamber. Suspensions of A549 and A549/DDP cells were prepared and added to the upper chamber of the Transwell chamber, while 600 μL of medium containing FBS was added to the lower chamber. After 24 hours of cultivation, the culture solution was discarded, and the cells were washed using PBS. Non-migrated cells in the upper chamber were gently removed using cotton swabs. A549 and A549/DDP cells were fixed using formaldehyde for 30 minutes and stained with 0.1% crystal violet staining solution for another 30 minutes. Subsequently, a cell count was performed.

Relationship between HLA-DPA1 and the immune microenvironment

We evaluated the immune microenvironment in LUAD tissues by calculating immune scores and relative expression levels of immune cells using both single-sample Gene Set Enrichment Analysis and estimate methods. Pearson correlation analysis was used to investigate the relationship between HLA-DPA1 expression and the assessed immune characteristics. Additionally, we divided the samples into two groups based on the median value of HLA-DPA1 and examined the differences in immune scores and immune cell levels between these groups.

Statistical analysis

We used the Wilcoxon rank sum test to determine the levels of HLA-DPA1 in LUAD tissues from TCGA, XENA, and GEO datasets. ROC, survival, and correlation analyses were employed to investigate the diagnostic value of HLA-DPA1 expression in LUAD, examine the relationship between HLA-DPA1 expression and patient prognosis, and analyze correlations between HLA-DPA1 expression and immune-related factors, respectively. Additionally, we used *t*-tests to evaluate the impact of HLA-DPA1 overexpression on cell proliferation, migration, and invasion. Data visualization and analysis were performed using Graph Prism software and the Xiantao Academic website (https://www.xiantaozi.com/products). For all statistical analyses, we considered a *P*-value of less than 0.05 statistically significant.

Data availability

The data was available in the TCGA (https://portal.gdc.cancer.gov/), GEPIA (http://gepia.cancer-pku.cn), UALCAN (http://ualcan.path.uab.edu), XENA (https://xena.ucsc.edu/), Xiantao Academic (https://www.xiantaozi.com/products), TIMER (https://cistrome.shinyapps.io/timer/), DAVID (https://david.ncifcrf.gov/), LCE (https://lce.biohpc.swmed.edu/), TISIDB (http://cis.hku.hk/TISIDB/), and GEO (https://www.ncbi.nlm.nih.gov/geo) databases.

AUTHOR CONTRIBUTIONS

Qiang Guo, Wei-Min Luo, and Jun Zhang conceived the study. Ke Shi, Qian-Yun Li, Huan Huang, and Jun Zhang designed and completed the experimental plan. Ke Shi, Qian-Yun Li, Yun-Qiang Zhang, Dong-Xiao Ding, and Huan Huang analyzed the cancer data in the databases. Ke Shi, Yun-Qiang Zhang, Qian-Yun Li, and Huan Huang wrote the manuscript, and all authors read and confirmed the final manuscript.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest related to this study.

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Editorial note

[&]This corresponding author has a verified history of publications using a personal email address for correspondence.

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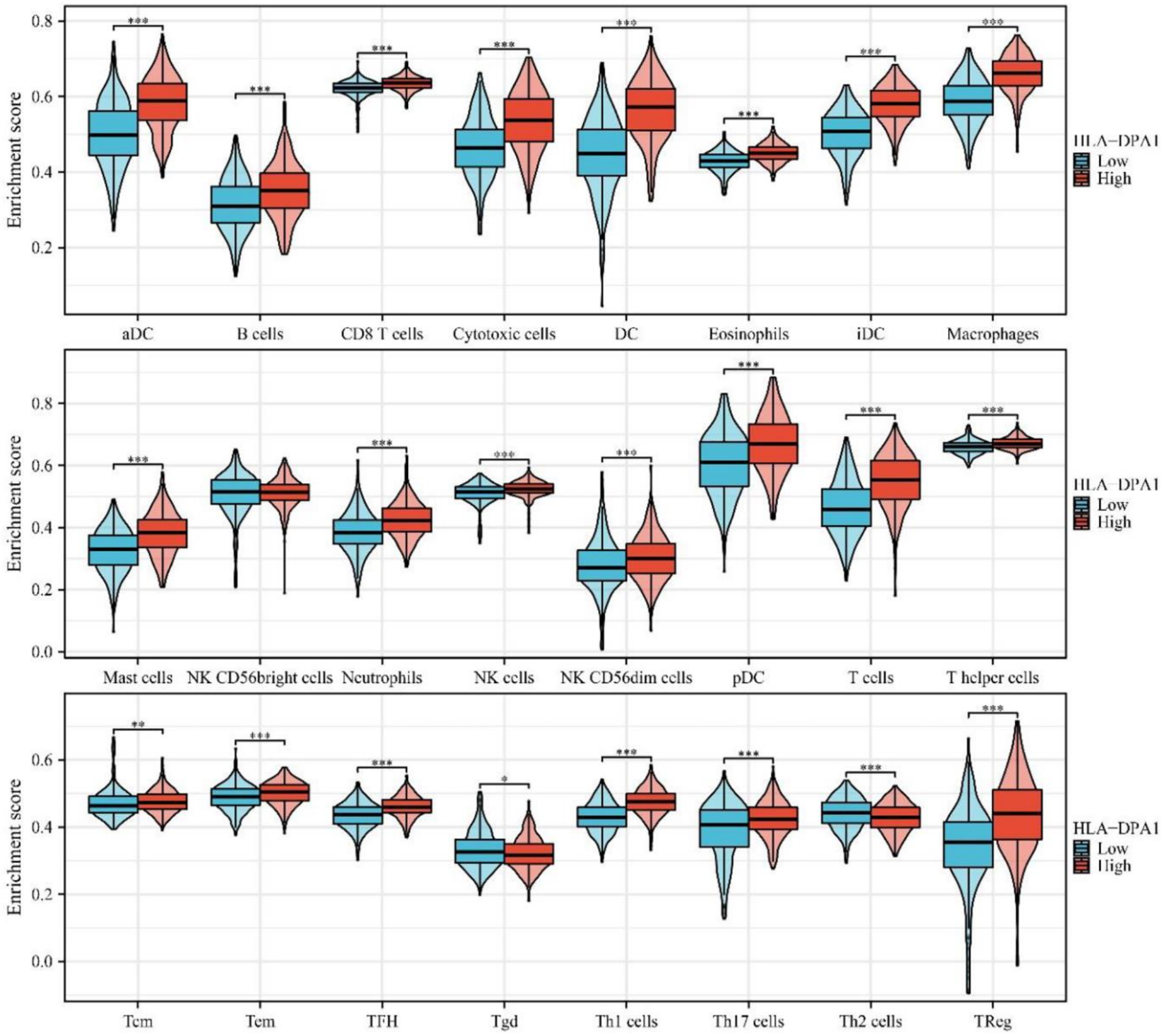
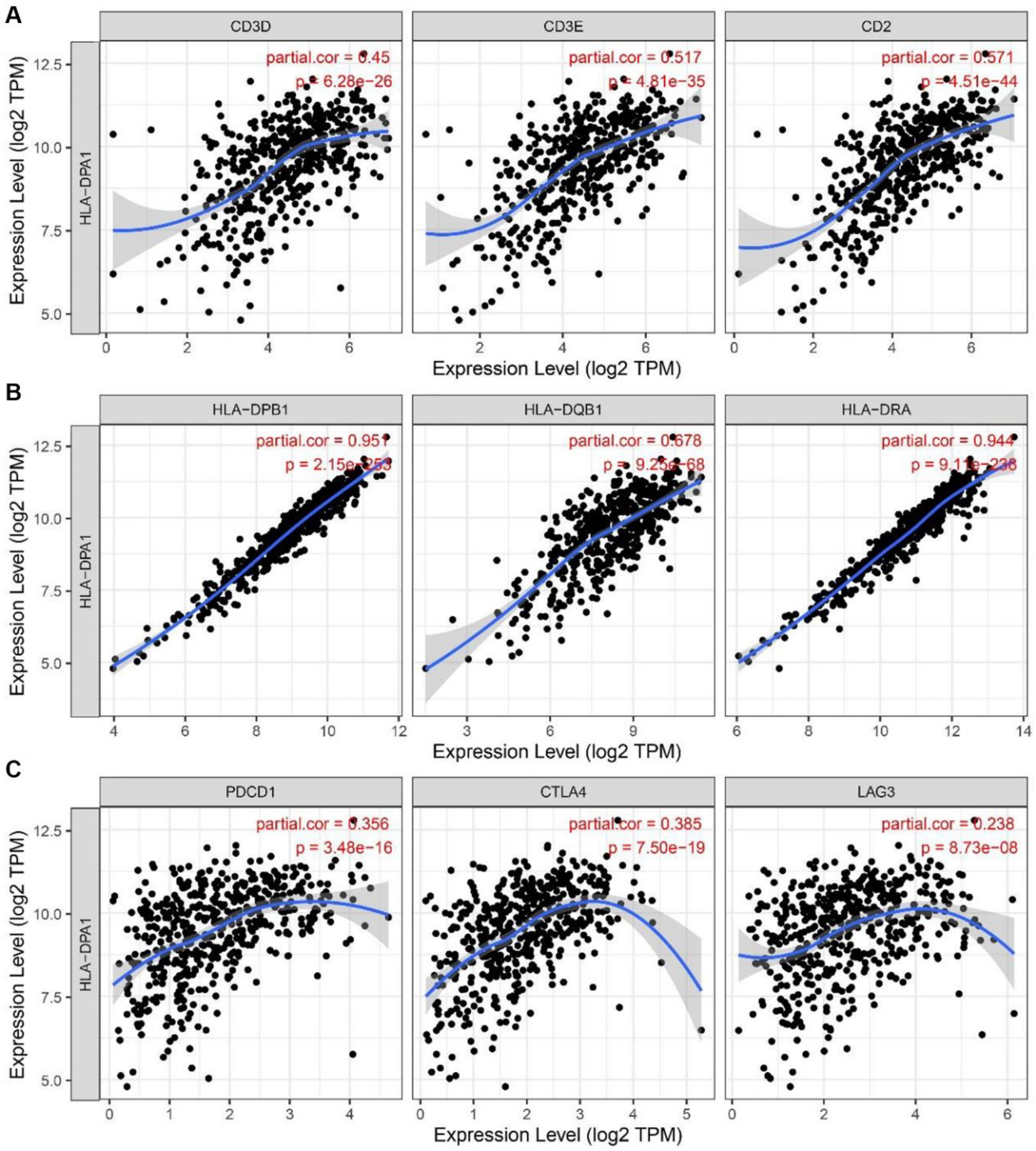
SUPPLEMENTARY MATERIALS

Supplementary Figures



Supplementary Figure 1. The immune cells in high- and low-HLA-DPA1 expression groups.



Supplementary Figure 2. The correlation between the HLA-DPA1 overexpression and immune cell markers in the context of tumor purity. (**A**) T cell markers; (**B**) Dendritic cell markers; (**C**) T cell exhaustion markers.

Supplementary Tables

Please browse Full Text version to see the data of Supplementary Table 2.

Supplementary Table 1. Genes positively co-expressed with HLA-DPA1.

Gene	Coefficient	Gene	Coefficient	Gene	Coefficient	Gene	Coefficient
HLA-DPA1	1	GIMAP2	0.709154362	IL10RA	0.665266461	B2M	0.629901396
HLA-DRA	0.939929272	IRF8	0.707679199	FGR	0.663879355	HVCN1	0.628828632
HLA-DPB1	0.923988098	FCER1G	0.707343115	IL16	0.662454606	APBB1IP	0.628491844
CD74	0.899900908	LCP1	0.706873538	RAC2	0.661552796	APOBEC3G	0.628477428
HLA-DMB	0.89516136	WAS	0.706465797	CD40	0.656945302	PLD4	0.628209624
HLA-DRB1	0.894503912	FOLR2	0.706257982	STX11	0.656687429	GNGT2	0.627258125
HLA-DOA	0.884142667	HCK	0.705229462	ADORA3	0.656329789	SLC7A7	0.62652732
HLA-DQA1	0.865189432	TLR7	0.70445824	VSIG4	0.655937383	TRAC	0.626121154
HLA-DMA	0.847758139	EVI2B	0.70403413	AOAH	0.655612225	PLAAT4	0.625453196
HLA-DRB5	0.81288267	HLA-B	0.703761948	GPR65	0.655318311	CXorf21	0.625009856
CD4	0.797077423	JAML	0.702048268	CCR5	0.65510061	HLA-DRB9	0.624574107
HLA-DQB1	0.796030118	GMFG	0.70015895	CMKLR1	0.654985087	CSF2RA	0.624137366
ITGB2	0.788547922	MPEG1	0.698885411	SIGLEC7	0.65427375	PIK3R6	0.623917277
LY86	0.785160735	SIGLEC9	0.698402631	ARHGDIB	0.653929457	PTPRO	0.623299519
MNDA	0.783246088	NAPSB	0.69803603	NCF2	0.652792325	SLC37A2	0.622955534
AIF1	0.772757553	C1QC	0.695624592	ALOX5AP	0.652546315	PTGER4	0.621546807
TNFAIP8L2	0.766436301	CD300C	0.695043028	APOL3	0.652251108	TRAV9-2	0.621295947
BTK	0.764783007	TREM2	0.694295369	CSF2RB	0.651149346	HPGDS	0.621078907
SASH3	0.759361863	ITGAM	0.693786636	BIN2	0.650272112	LAT2	0.619846708
RNASE6	0.758584752	LCP2	0.693319357	CCR2	0.649746926	PTAFR	0.619758083
LST1	0.757025543	C1QB	0.693105046	FCGR2A	0.649034746	GIMAP7	0.619382155
SNX20	0.756369058	ABI3	0.69262654	TESPA1	0.648898786	GIMAP1	0.618293486
SELPLG	0.75520627	CD83	0.69206892	CPVL	0.648893369	DPYD	0.617323461
CASP1	0.754163424	P2RX7	0.691813207	MSR1	0.648375003	LIPA	0.61652342
HLA-E	0.753729746	CTSS	0.691611696	TRBV20-1	0.647268782	SUSD3	0.61648575
LAPTM5	0.753479911	GIMAP4	0.688455211	SIGLEC1	0.645604697	GAPT	0.61581821
MS4A6A	0.752081068	P2RY12	0.687804712	STAT5A	0.645207313	CTSO	0.615547705
NCKAP1L	0.751531026	ARHGEF6	0.684457645	GIMAP6	0.644978996	GPR34	0.615511763
NCF4	0.750258443	C1QA	0.683927379	PIK3AP1	0.64456783	SLC9A9	0.612763332
CSF1R	0.749004745	FPR3	0.683479884	TRPV2	0.643274177	PTPN7	0.612669918
CD33	0.745531105	PILRA	0.683236633	RASGRP4	0.642443942	GAB3	0.611165095
LAIR1	0.745137597	TRIM22	0.682640635	PPM1M	0.64024745	SIRPA	0.610996844
DOK2	0.743745445	PLEKHO2	0.68236022	CD2	0.639726955	BTN2A2	0.610550918
IGSF6	0.742384755	TNFSF13B	0.682305822	MS4A7	0.639638351	CD14	0.610491259
HAVCR2	0.741026835	FERMT3	0.681442247	VAV1	0.639063669	TNFRSF1B	0.609933332
SPN	0.740607311	CARD16	0.680719773	ARHGAP25	0.638706387	SOWAHD	0.60992759
PLEK	0.739850317	MYO1F	0.680453501	PIK3R5	0.638646893	NLRP3	0.609720717
C1orf162	0.736860456	MS4A4A	0.679303416	FGL2	0.637921546	SIRPB2	0.609692421
CD53	0.734130409	ARHGAP30	0.678961328	IL12RB1	0.637371434	GLIPR2	0.608938195
SPI1	0.733722089	CYTH4	0.678864793	CD48	0.637292106	LYZ	0.607803012
CIITA	0.733256993	SLAMF8	0.67822393	DPEP2	0.636755608	PLXNC1	0.606206182

CLEC10A	0.7311992	TMEM273	0.676954861	FCGR3A	0.636637542	RGS18	0.605792372
NFAM1	0.728820941	LRRC25	0.676003165	IKZF1	0.636397279	ARHGAP15	0.605765121
P2RY13	0.728515817	DOCK2	0.675190718	RTN1	0.635615729	PIK3CG	0.605572854
LPXN	0.728140858	FGD2	0.675172682	C16orf54	0.634502003	ACP5	0.605251514
HLA-DQB2	0.726972607	TLR2	0.674083278	FAM78A	0.634174009	RCSD1	0.604741018
CD300LF	0.726079309	BTN3A3	0.673399207	OLR1	0.633164644	APOBEC3C	0.604032018
C3AR1	0.726078036	TLR8	0.672881567	OSCAR	0.632783049	CD1C	0.603010184
CD86	0.725319008	PARVG	0.671433867	DOCK8	0.631741926	ARHGAP9	0.602774842
GGTA1	0.723482082	PTPRC	0.670614401	FCGR1A	0.631655081	GPNMB	0.602568483
CYBB	0.722112758	TAGAP	0.670340181	SLA	0.631621015	HCST	0.602329332
SLC15A3	0.720823718	LSP1	0.670221883	CRTAM	0.631082981	TRBV19	0.601083604
CD37	0.718415678	ALOX5	0.669901347	HLA-F	0.630999906	NRROS	0.600937344
CD52	0.71295562	ITGAL	0.668225928	LILRB1	0.630278903	NCF1	0.600869284
SCIMP	0.711263816	CD84	0.6679874	MRC1	0.630169002	PSTPIP1	0.6008261
SLCO2B1	0.710385258	HLA-DOB	0.667899962	CLEC7A	0.630093914	LGALS9	0.600292892
TYROBP	0.709641209	SAMHD1	0.666470219	PTPN22	0.630090261	TRAV8-2	0.600056566
CLECL1	0.6000455						

Supplementary Table 2. The biological functions of genes co-expressed with HLA-DPA1 using GO analysis in the DAVID database.

Supplementary Table 3. The biological functions of HLA-DPA1 using GO analysis in the TISIDB database.

GO type	Term
BP	GO:0001819 positive regulation of cytokine production
BP	GO:0002429 immune response-activating cell surface receptor signaling pathway
BP	GO:0002478 antigen processing and presentation of exogenous peptide antigen
BP	GO:0002495 antigen processing and presentation of peptide antigen via MHC class II
BP	GO:0002504 antigen processing and presentation of peptide or polysaccharide antigen via MHC class II
BP	GO:0002694 regulation of leukocyte activation
BP	GO:0002696 positive regulation of leukocyte activation
BP	GO:0002757 immune response-activating signal transduction
BP	GO:0002764 immune response-regulating signaling pathway
BP	GO:0002768 immune response-regulating cell surface receptor signaling pathway
BP	GO:0007159 leukocyte cell-cell adhesion
BP	GO:0019882 antigen processing and presentation
BP	GO:0019884 antigen processing and presentation of exogenous antigen
BP	GO:0019886 antigen processing and presentation of exogenous peptide antigen via MHC class II
BP	GO:0022407 regulation of cell-cell adhesion
BP	GO:0022409 positive regulation of cell-cell adhesion
BP	GO:0031294 lymphocyte costimulation
BP	GO:0031295 T cell costimulation
BP	GO:0032609 interferon-gamma production
BP	GO:0032649 regulation of interferon-gamma production
BP	GO:0032729 positive regulation of interferon-gamma production
BP	GO:0032943 mononuclear cell proliferation
BP	GO:0032944 regulation of mononuclear cell proliferation

BP	GO:0032946 positive regulation of mononuclear cell proliferation
BP	GO:0034341 response to interferon-gamma
BP	GO:0042098 T cell proliferation
BP	GO:0042102 positive regulation of T cell proliferation
BP	GO:0042110 T cell activation
BP	GO:0042129 regulation of T cell proliferation
BP	GO:0045785 positive regulation of cell adhesion
BP	GO:0046651 lymphocyte proliferation
BP	GO:0048002 antigen processing and presentation of peptide antigen
BP	GO:0050670 regulation of lymphocyte proliferation
BP	GO:0050671 positive regulation of lymphocyte proliferation
BP	GO:0050851 antigen receptor-mediated signaling pathway
BP	GO:0050852 T cell receptor signaling pathway
BP	GO:0050863 regulation of T cell activation
BP	GO:0050865 regulation of cell activation
BP	GO:0050867 positive regulation of cell activation
BP	GO:0050870 positive regulation of T cell activation
BP	GO:0051249 regulation of lymphocyte activation
BP	GO:0051251 positive regulation of lymphocyte activation
BP	GO:0060333 interferon-gamma-mediated signaling pathway
BP	GO:0070486 leukocyte aggregation
BP	GO:0070489 T cell aggregation
BP	GO:0070661 leukocyte proliferation
BP	GO:0070663 regulation of leukocyte proliferation
BP	GO:0070665 positive regulation of leukocyte proliferation
BP	GO:0071346 cellular response to interferon-gamma
BP	GO:0071593 lymphocyte aggregation
BP	GO:1903037 regulation of leukocyte cell-cell adhesion
BP	GO:1903039 positive regulation of leukocyte cell-cell adhesion
MF	GO:0003823 antigen binding
MF	GO:0032395 MHC class II receptor activity
MF	GO:0033218 amide binding
MF	GO:0042277 peptide binding
MF	GO:0042605 peptide antigen binding
CC	GO:0005765 lysosomal membrane
CC	GO:0005802 trans-Golgi network
CC	GO:0010008 endosome membrane
CC	GO:0012507 ER to Golgi transport vesicle membrane
CC	GO:0030133 transport vesicle
CC	GO:0030134 ER to Golgi transport vesicle
CC	GO:0030135 coated vesicle
CC	GO:0030136 clathrin-coated vesicle
CC	GO:0030139 endocytic vesicle
CC	GO:0030176 integral component of endoplasmic reticulum membrane
CC	GO:0030658 transport vesicle membrane
CC	GO:0030659 cytoplasmic vesicle membrane
CC	GO:0030662 coated vesicle membrane

CC	GO:0030665 clathrin-coated vesicle membrane
CC	GO:0030666 endocytic vesicle membrane
CC	GO:0030669 clathrin-coated endocytic vesicle membrane
CC	GO:0031227 intrinsic component of endoplasmic reticulum membrane
CC	GO:0031984 organelle subcompartment
CC	GO:0032588 trans-Golgi network membrane
CC	GO:0042611 MHC protein complex
CC	GO:0042613 MHC class II protein complex
CC	GO:0044440 endosomal part
CC	GO:0045334 clathrin-coated endocytic vesicle
CC	GO:0071556 integral component of lumenal side of endoplasmic reticulum membrane
CC	GO:0098552 side of membrane
CC	GO:0098553 lumenal side of endoplasmic reticulum membrane
CC	GO:0098791 Golgi subcompartment
CC	GO:0098852 lytic vacuole membrane

Abbreviations: GO: gene ontology; BP: biological process; MF: molecular function; CC: cellular component.