Journal of Inflammation Research

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

HDAC1 Mediates Immunosuppression of the Tumor Microenvironment in Non-Small Cell Lung Cancer

Yongfei Fan[1,2,*], Xiang Ji[3,*], Kai Yuan[1,2], Qiyong Wu[1,2], Ming Lou[1,2]

[1]Department of Thoracic Surgery, The second People's Hospital of Changzhou, the Third Affiliated Hospital of Nanjing Medical University, Changzhou, 213164, People's Republic of China; [2]Heart and Lung Disease Laboratory, The second People's Hospital of Changzhou, the Third Affiliated Hospital of Nanjing Medical University, Changzhou, 213164, People's Republic of China; [3]Department of Thoracic Surgery, The First Affiliated Hospital of Bengbu Medical University, Bengbu, 233000, People's Republic of China

*These authors contributed equally to this work

Correspondence: Ming Lou; Qiyong Wu, Department of Thoracic Surgery, The second People's Hospital of Changzhou, the Third Affiliated Hospital of Nanjing Medical University, Changzhou, 213164, People's Republic of China, Email helloloum@163.com; wqyxycxy@aliyun.com

Background: Studies have demonstrated that histone deacetylase 1 (*HDAC1*) enables cancer cells to evade killing mediated by cytotoxic T lymphocytes. However, there are no studies on the immunological aspects of *HDAC1* in non-small cell lung cancer (NSCLC).

Methods: In this research, we used the Cancer Genome Atlas (TCGA) public database combined with tissue microarray (TMA) to investigate *HDAC1* expression and prognosis in NSCLC. According to the median value of *HDAC1* expression in the TCGA dataset, samples of patients with NSCLC were classified into high- and low-expression cohorts. Subsequently, the biological characteristics of *HDAC1* in high- and low-expression cohorts in terms of signaling pathways, immune cell infiltration, immune cell function, and genomic stability were investigated to better understand the effect of *HDAC1* in the tumor microenvironment (TME) of NSCLC. Additionally, we selected tissue samples with *HDAC1* overexpression in TMA2 and performed immunohistochemical staining of $CD8^+$ T cells to observe the distribution of $CD8^+$ T cells in the tumor.

Results: The findings revealed that overexpression of *HDAC1* in NSCLC was associated with poor prognosis. Analysis of signaling pathway enrichment indicated that *HDAC1* downregulated immune-related signaling pathways in NSCLC. Immune cell infiltration, immune cell function, and genomic stability analyses suggested that the TME alteration mediated by *HDAC1* in the high-expression cohort was consistent with the "immune desert" phenotype. Furthermore, $CD8^+$ T immunohistochemical staining experiments of tissue samples with *HDAC1* overexpression in NSCLC revealed few $CD8^+$ T cells distributed in the tumor parenchyma and interstitium.

Conclusion: Conclusively, our findings from several biological analyses revealed that *HDAC1* is overexpressed in NSCLC and induces TME immunosuppression.

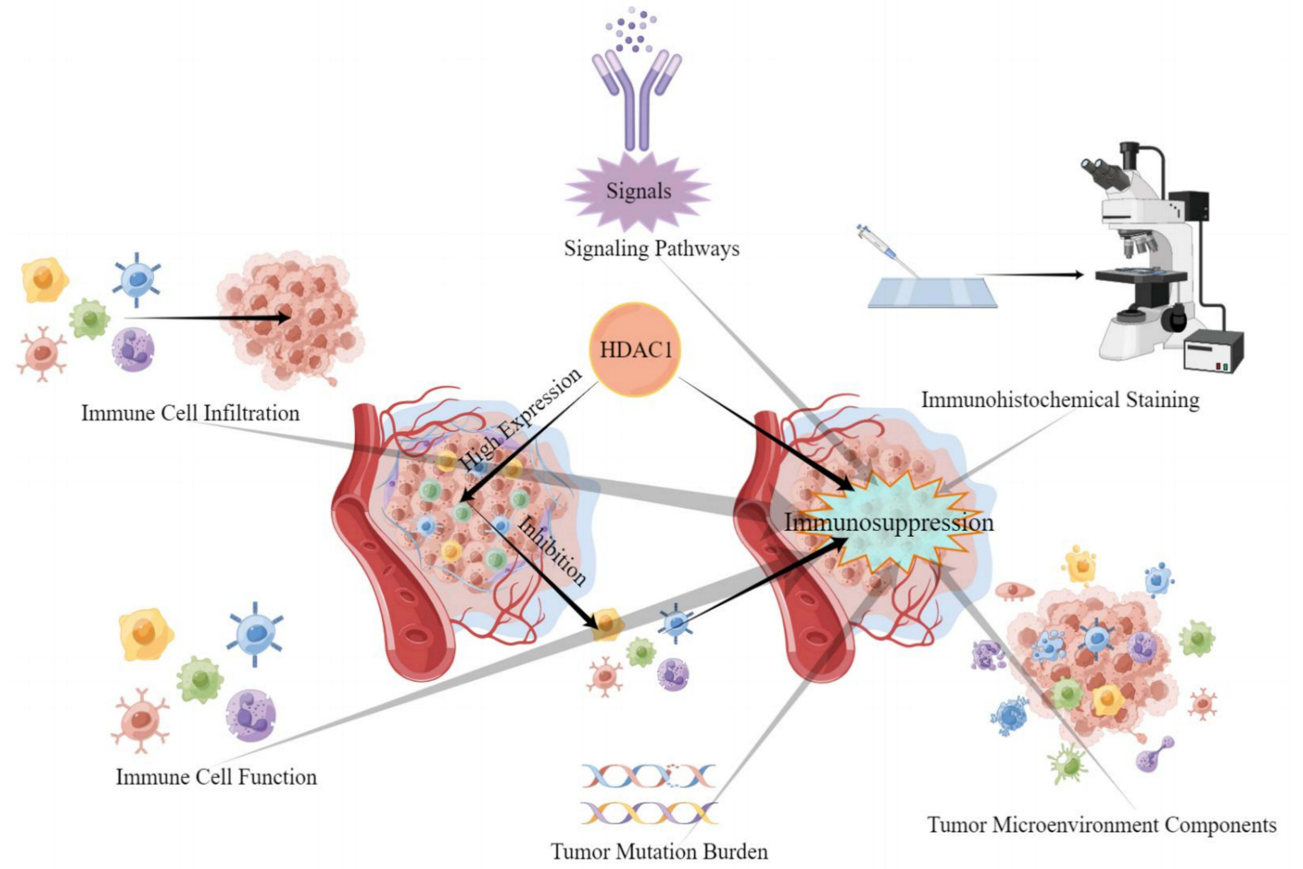
Keywords: *HDAC1*, non-small cell lung cancer, immunosuppression, prognosis, tumor microenvironment

Introduction

Lung cancer is the most common malignant tumor of the respiratory system and poses a major threat to human health. According to statistics, every year, more than 2 million new cases of lung cancer are diagnosed, and 1.76 million people die from it worldwide.[1] The most common subtype of lung cancer, non-small cell lung cancer (NSCLC), which comprises lung squamous carcinoma (LUSC) and lung adenocarcinoma (LUAD) subtypes, accounts for 85% of newly diagnosed cases each year.[2] The average 5-year survival rate of patients with lung cancer is <20% owing to the highly insidious and aggressive nature of lung cancer, which causes some patients to be diagnosed at a late stage.[3,4] Therefore, establishing effective treatments to improve the prognosis of NSCLC is required.

Received: 29 November 2024
Accepted: 13 February 2025
Published: 8 March 2025

Graphical Abstract



Immunotherapy by enhancing autoimmunity against tumor cells is a breakthrough in tumor treatment, particularly in NSCLC.[5,6] Immune checkpoint inhibitors (ICIs), mainly programmed cell death receptor 1 (*PD-1*), PD-ligand 1 (*PD-L1*), and cytotoxic T-lymphocyte-associated protein 4 receptor (*CTLA4*) have significantly improved the survival time and quality of life of patients with advanced NSCLC.[7,8] However, we observed unsatisfactory immunotherapy outcomes in some patients with NSCLC during long-term clinical treatment.

The tumor microenvironment (TME) is the microenvironment surrounding tumor cells. It is a complex integrated system of immune cells, extracellular matrix, bone marrow-derived inflammatory cells, surrounding blood vessels, fibroblasts and signaling molecules.[9,10] Immune cells in the TME and how they are modulated play an essential role in tumorigenesis and progression, and TME can influence the patient's response to immune cells.[9,11,12] TME can be classified into "immune desert", "immune inflammation", and "immune evasion" phenotypes.[12] Patients who fit into the "immune desert" and "immune evasion" phenotypes are less responsive to immunotherapy than those who fit into the "immune inflammation".[12,13] Therefore, the targeted TME therapy approach has provided new prospects for cancer treatment in recent years.[11,14]

The protein encoded by histone deacetylase 1 (*HDAC1*) is a member of the HDAC family and is a component of the HDAC complex. *HDAC1* catalyzes the removal of acetyl cohorts from lysine residues of histone and non-histone substrates, causing transcriptional repression.[15] In the nucleus, histone acetylation and deacetylation are in dynamic equilibrium, and eukaryotic gene expression is co-regulated by histone acetyltransferase and HDAC.[15] Studies have demonstrated that HDACs participate in the regulation of cell biological processes, such as cell cycle, angiogenesis, immune regulation, and DNA damage response.[15,16] Previous studies have reported that *HDAC1* knockdown in NSCLC inhibits tumor cell proliferation, tumor neoangiogenesis,

invasion, and migration and promotes apoptosis.[17] Interestingly, it also has a role in promoting NSCLC invasion and migration in a hypoxic environment.[18] Additionally, genome-wide Clustered Regularly Interspaced Short Palindromic Repeats screen conducted by Lawson et al, revealed that *HDAC1* enables cancer cells to evade killing mediated by cytotoxic T lymphocytes.[19]

In this research, we will investigate the biological roles of *HDAC1* expression in the immunological aspects of TME in NSCLC and seek new directions to improve the effectiveness of immunotherapy.

Materials and Methods

Acquisition and Analysis of HDAC1 Expression Profiles in Public Databases

The pan-cancer dataset (tumor = 10363; normal = 730) from the Cancer Genome Atlas (TCGA; https://www.cancer.gov/) public database were selected using the Sento Academic (https://www.xiantao.love/) online website to compare the unpaired differential expression of *HDAC1* in tumor and normal tissues of 33 cancers. NSCLC was selected as the target of this study. Then, the transcriptomic dataset of *HDAC1* in NSCLC (tumor = 1037; normal = 108) and files containing the clinicopathological parameters (including follow-up time, age, sex, total pathological stage, T stage, N stage and M stage) were retrieved from the TCGA database. Table 1 demonstrates the specific information of patients included in the TCGA dataset in this study. Analysis of paired and unpaired differential expression of *HDAC1* in NSCLC tumor tissues and normal tissues in the TCGA dataset was completed using the "limma" R package.

Table 1 Clinical Information of Patients With NSCLC in TCGA and TMA Datasets

Clinical Information		TCGA (n = 1037)	TMA1 (n = 60)	TMA2 (n = 140)
Sex	Female	417 (40.2%)	18 (31.6%)	28 (20.0%)
	Male	620 (59.8%)	39 (68.4%)	112 (80.0%)
Age	<=65	446 (43.0%)	34 (59.6%)	92 (65.7%)
	>65	563 (54.3%)	23 (40.4%)	48 (34.3%)
	Missing	28 (2.7%)	0 (0.0%)	0 (0.0%)
Stage	I	539 (52.0%)	32 (56.1%)	67 (47.9%)
	II	285 (27.5%)	6 (10.5%)	44 (31.4%)
	III	168 (16.2%)	5 (8.8%)	29 (20.7%)
	IV	33 (3.2%)	14 (24.6%)	0 (0.0%)
	Missing	0 (0.0%)	0 (0.0%)	0 (0.0%)
T stage	T1	289 (27.9%)	34 (59.6%)	15 (10.7%)
	T2	583 (56.2%)	13 (22.8%)	105 (75.0%)
	T3	120 (11.6%)	8 (14.0%)	20 (14.3%)
	T4	42 (4.1%)	2 (3.5%)	0 (0.0%)
	Missing	3 (0.3%)	0 (0.0%)	0 (0.0%)
N stage	N0	668 (64.4%)	48 (84.2%)	82 (58.6%)
	N1	226 (21.8%)	5 (8.8%)	31 (22.1%)
	N2	114 (11.0%)	0 (0.0%)	27 (19.3%)
	N3	7 (0.7%)	4 (7.0%)	0 (0.0%)
	Missing	22 (2.1%)	0 (0.0%)	0 (0.0%)
M stage	M0	773 (74.5%)	43 (75.4%)	140 (100.0%)
	M1	32 (3.1%)	14 (24.6%)	0 (0.0%)
	Missing	232 (22.4%)	0 (0.0%)	0 (0.0%)
Follow-up time (day)	Range	30–7248	——	90–3030
Survival status	Death	381 (36.7%)	——	91 (65.0%)
	Alive	594 (57.3%)	——	49 (35.0%)
	Missing	0 (0.0%)	——	0 (0.0%)

Construction of Tissue Microarray

A total of 60 pairs of NSCLC tissue and paracancerous tissue samples were used for tissue microarrays (TMA) 1 were purchased from Superbiotek Biologicals (ZL-lug1201; China). Paired difference expression analysis of *HDAC1* in NSCLC tumor tissues and paraneoplastic tissues was performed in this experiment using TMA1. Tissue samples for TMA2 were collected from patients who underwent surgical resection in thoracic surgery from January to December 2005 (Zhongshan Hospital, Fudan University; China). TMA2 was composed of 140 NSCLC tissues and 10 randomly obtained normal tissues, and performed unpaired differential expression analysis of *HDAC1* in NSCLC tumor tissues and normal tissues. Moreover, complete information on postoperative follow-up time (follow-up to July 2013) and clinicopathological parameters were available for the 140 patients with NSCLC. Table 1 demonstrates detailed information of patients with NSCLC samples in TMA1 and TMA2. All patients included in this study did not receive targeted therapy, radiation therapy, or chemotherapy preoperatively, and the patients were anonymized per international regulations.

Immunohistochemical Staining and Quantitative Analysis

To observe the expression level of *HDAC1* protein in NSCLC tumor tissues and normal tissues by using immunohistochemical indirect staining. The specific steps were as follows:

Paraffin specimens were cut to 4 μm thickness and mounted on slides for dewaxing and rehydration operations, followed by antigen repair. On the treated slides, 10% normal goat serum was added dropwise. It was closed at room temperature (25 °C) for 60 minutes and then incubated overnight at 4 °C with the primary antibody (1:50, ab53091, Abcam, UK). The following day, the primary antibody was rinsed off, and the slides were washed twice with a phosphate-buffered salt solution for 10 minutes. Subsequently, the secondary antibody (1:250, RGAR011, Proteintech, China) was added to the slides, and the slides were incubated for 30 minutes at room temperature (25 °C), cleaned, and washed twice with phosphate buffer solution for 10 minutes each time. Furthermore, a drop of diaminobenzidine solution was added to the slides for wet box incubation and the slides were re-stained with the experimental step of hematoxylin at 37 °C for 5 minutes. Finally, the slides were dehydrated with 50%, 75%, 85%, and 95% alcohol, and sealed with 1 drop of neutral gel.

The slides were captured for examination using an optical inverted microscope, and the original section images were captured under the microscope. To quantify *HDAC1* protein level expression in NSCLC, we used Image J (version: 1.8.0_172) software to convert *HDAC1* protein staining intensity in immunohistochemically stained sections into average optical density (AOD) values. The equation for AOD calculation is as follows:

$$\mathrm{AOD} = \frac{\text{Staining area optical density}}{\text{Staining area}}$$

Finally, the expression of *HDAC1* extracted by Image J in TMA1 and TMA2 was analyzed for paired and unpaired differences using GraphPad Prism software (version: 9.4.1.681).

Prognostic Analysis of HDAC1 in NSCLC

The "Auto select best cutoff" module was selected through the Kaplan Meier plotter database (https://kmplot.com/analysis/) to classify low (n = 1267) and high (n = 658) expression cohorts according to *HDAC1* expression in NSCLC (Affymetrix ID: 201209_at) and to compare their overall survival (n = 1925) differences between two cohorts. Subsequently, the expression data of *HDAC1* in TMA2 were integrated with the survival follow-up data. The median value of *HDAC1* expression was utilized to classify the patient samples into low- and high-expression cohorts, and Kaplan Meier survival curves were plotted by GraphPad Prism software. Additionally, we used TCGA dataset to integrate *HDAC1* expression levels with clinicopathological parameters (including follow-up time, age, sex, total pathological stage, T stage, N stage and M stage) to construct a nomogram survival prediction scoring system and predictive calibration curves using the "rms" R language package. Each patient's clinicopathological stage was assigned a score, and the scores of all clinical parameters were summed to obtain the patient's overall score. Based on the patient's overall score, the survival rates of patients with NSCLC were predicted.[20]

Evaluation of the Immunological Role of HDAC1 in NSCLC

To further investigate the relationship between *HDAC1* and NSCLC carcinogenesis, we performed gene set enrichment analysis (GSEA) to search for signaling pathways in which *HDAC1* may be involved. Furthermore, TCGA dataset was categorized into low- (n = 518) and high- (n = 519) expression cohorts on the basis of the median value (cutoff value = 38.37) of *HDAC1* expression in NSCLC. We downloaded the Hallmark gene set files from the Molecular Signatures Database[21] (http://www.gsea-msigdb.org/gsea/login.jsp), and performed enrichment analysis of *HDAC1* low- and high-expression cohorts on the Hallmark gene set by Gene Set Variation Analysis (GSVA) using the "GSVA" R language package. Additionally, immune cells and functional enrichment in *HDAC1* low - and high-expression cohorts were analyzed by single-sample GSEA (ssGSEA) method using the "GSVA" R language package.

The immune cell, tumor cell, and stromal cell components in the TME of NSCLC were compared in both the low- and high- expression cohorts of *HDAC1* by using the "estimateScore" function. This function used the estimation of stromal and immune cells in malignant tumor tissues using expression data (ESTIMATE) algorithm. Tumor Immune System Interactions Database (TISIDB; http://cis.hku.hk/TISIDB/index.php) was utilized to explore the relationship between *HDAC1* expression in NSCLC and lymphocytes.

In addition, patient tissues in TMA2 were categorized into high and low expression groups based on the AOD values of *HDAC1* expression. $CD8^+$ T cell (1:50, ab101500, UK) immunohistochemical staining was conducted on tissue samples from both groups to observe differences in $CD8^+$ T cell infiltration between the high and low *HDAC1* expression groups.

Statistical Analysis

In this study, the statistical analysis of the data was performed using GraphPad Prism software (version: 9.4.1.681) and R software (version: 4.1.1). The Log rank test method was used to make comparisons of the data between the two cohorts, and the Student's *t*-test method was employed to evaluate the statistical significance of normally distributed variables for quantitative data. P value < 0.05 was considered statistically significant.

Results

HDAC1 is Overexpressed in NSCLC

Differential expression analysis in pan-cancer indicated that *HDAC1* was expressed at significantly higher levels in tumor tissues than in normal tissues in various cancer types, including LUAD and LUSC (Figure 1A; P-value < 0.05). Analysis of the NSCLC dataset integrating LUAD and LUSC obtained from TCGA database revealed that *HDAC1* was expressed in tumor tissues at significantly higher levels than in normal tissues in both paired difference and unpaired difference analyses (Figure 1B and C; P-value < 0.05). Additionally, our findings were further confirmed by immunohistochemical staining experiments. Paired difference analysis in TMA1 and unpaired difference analysis in TMA2 demonstrated that *HDAC1* was expressed at significantly higher levels in tumor tissues than in normal tissues (Figure 1D–L; P-value < 0.05). Overall, *HDAC1* is overexpressed in NSCLC.

Overexpression of HDAC1 in NSCLC is Correlated with Poor Prognosis

Kaplan Meier plotter online website survival analysis suggested that the *HDAC1* high-expression cohort (cutoff value = 3013) had significantly worse survival time than the low-expression cohort (Figure 2A; P-value < 0.05). Additionally, the high-expression cohort, which was classified according to the median value (cutoff value = 0.158) of *HDAC1* expression in TMA2, also had significantly lower survival time compared to the low-expression cohort (Figure 2B; P-value < 0.05). Therefore, analysis of public databases combined with clinical follow-up data demonstrated that overexpression of *HDAC1* in NSCLC was correlated with poor prognosis.

The prognostic prediction accuracy of TCGA database is higher than that of TMA2 since it contains a larger sample of patients with NSCLC. Therefore, we used TCGA dataset to integrate *HDAC1* expression data with pathological parameters to construct a nomogram survival prediction scoring system to predict 1-, 3-, and 5-year survival rates of

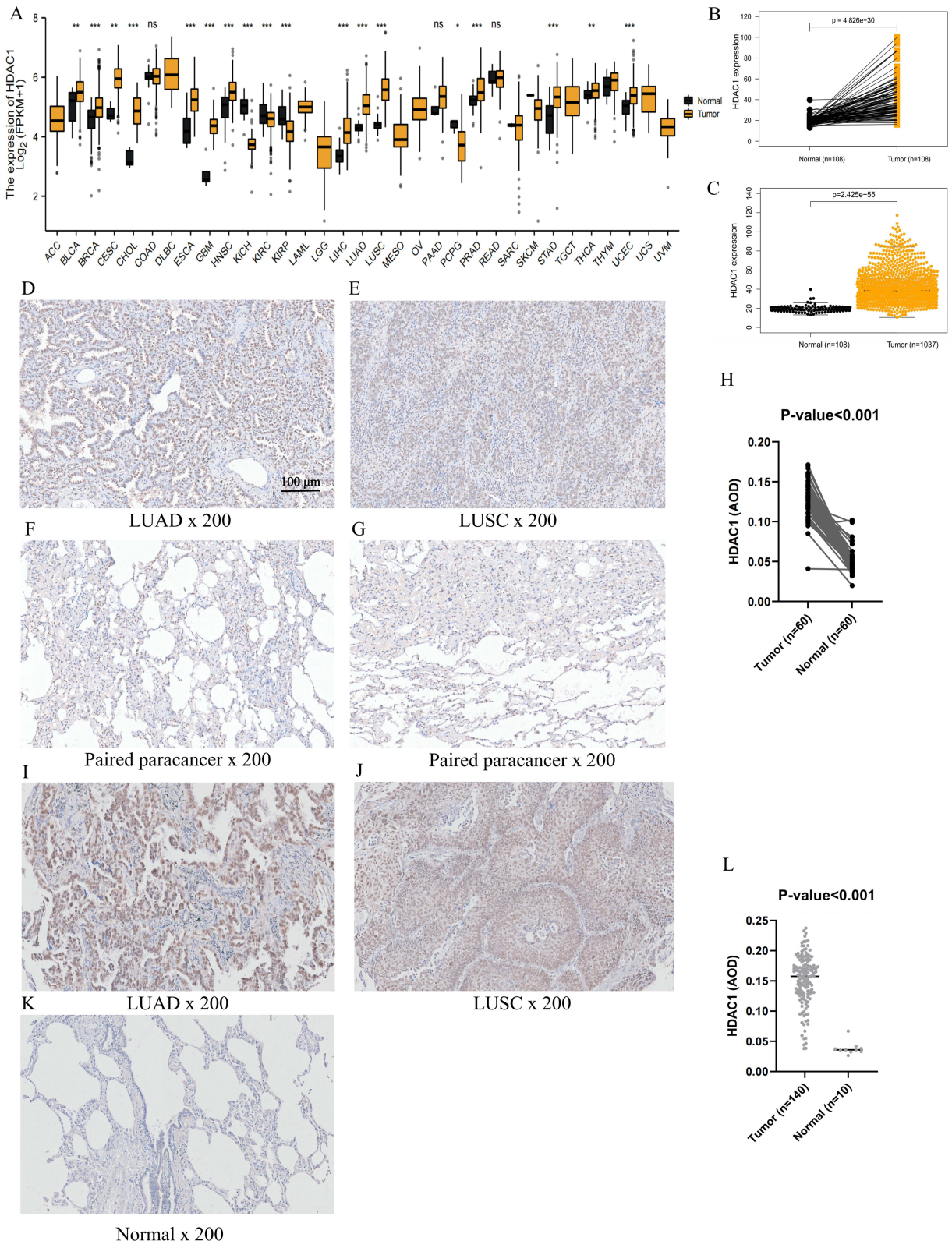


Figure 1 Analysis of HDAC1 expression in NSCLC. (**A**) Differential expression analysis of HDAC1 in pan-cancer. (**B**) Paired and (**C**) unpaired differential expression of HDAC1 in NSCLC. Immunohistochemical staining experiments were performed for (**D–H**) paired and (**I–L**) unpaired differential analysis of HDAC1 differential expression in tumor tissues versus normal tissues *P-value < 0.05; **P-value < 0.01; ***P-value < 0.001; ns: not significant.

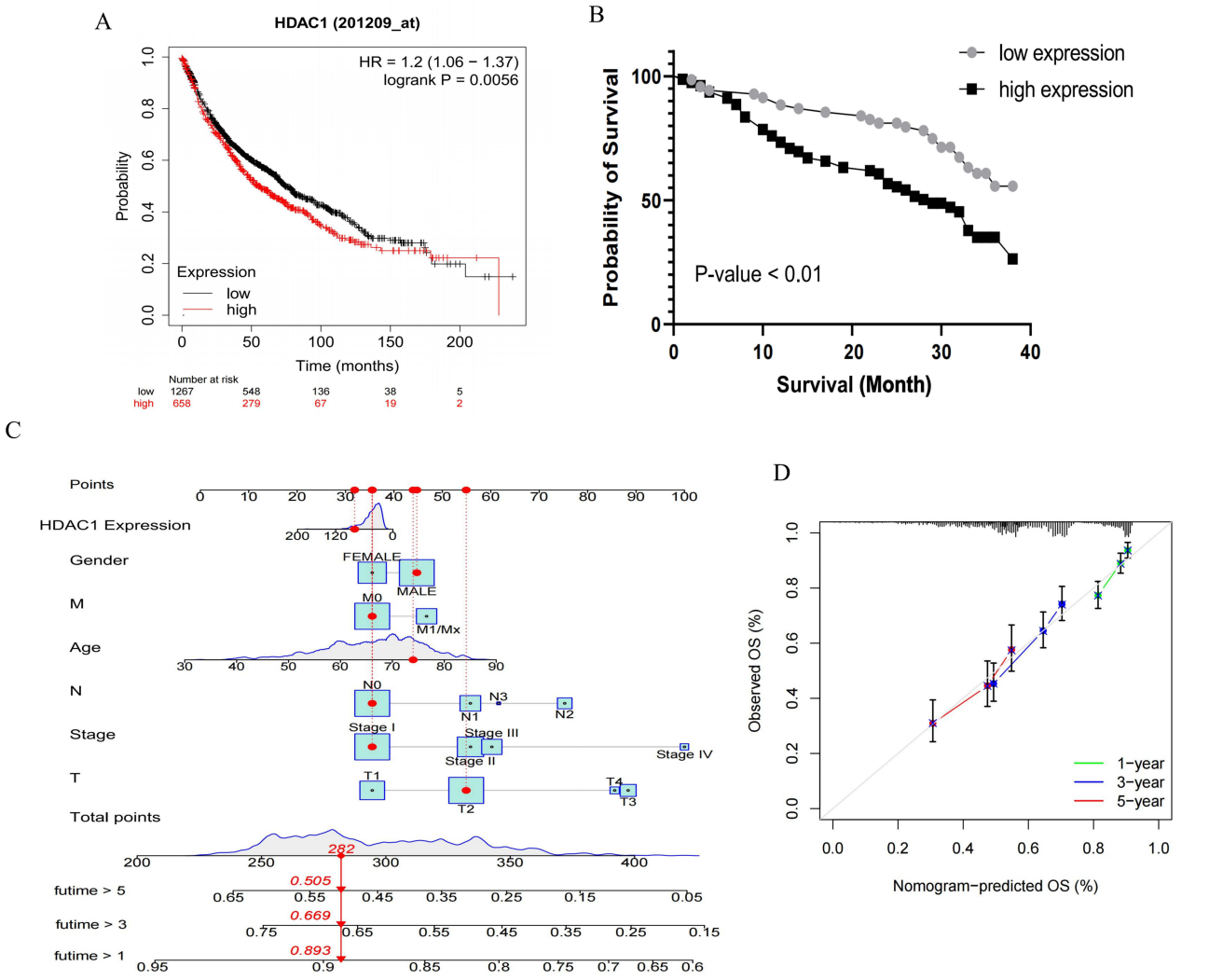


Figure 2 Prognostic analysis and prognosis prediction of HDAC1 in NSCLC. (**A**) Survival analysis of HDAC1 in NSCLC in Kaplan–Meier plotter database. (**B**) Combining clinical follow-up data with HDAC1 expression data in TMA for survival analysis. (**C**) Combining HDAC1 expression with clinicopathological parameters to construct a nomogram survival prediction system to predict 1-, 3-, and 5-year survival of patients. (**D**) Calibration curves to assess the predictive accuracy of the nomogram survival prediction system.

patients (Figure 2C). Furthermore, the prediction curves of 1-, 3-, and 5-year survival rates of the nomogram system fluctuated slightly above and below the calibration curve, indicating that the constructed prediction system had a high accuracy (Figure 2D).

HDAC1 Down-Regulates Immune Signaling Pathways in NSCLC

To further explore the involvement of *HDAC1* in the oncogenic mechanism of NSCLC, we performed a GSEA. The findings indicated that *HDAC1* down-regulates immune response-related signaling pathways in NSCLC, such as Fc epsilon RI, Janus kinase (JAK) /signal transducers and activators of transcription (STAT), peroxisome proliferator-activated receptor (PPAR), chemokine, nucleotide-binding oligomerization domain (NOD)-like receptor, Toll-like receptor, and B and T cell receptor signaling pathways (Figure 3). Therefore, we speculate that *HDAC1* may mediate immunosuppression in NSCLC.

https://doi.org/10.2147/JIR.S509316

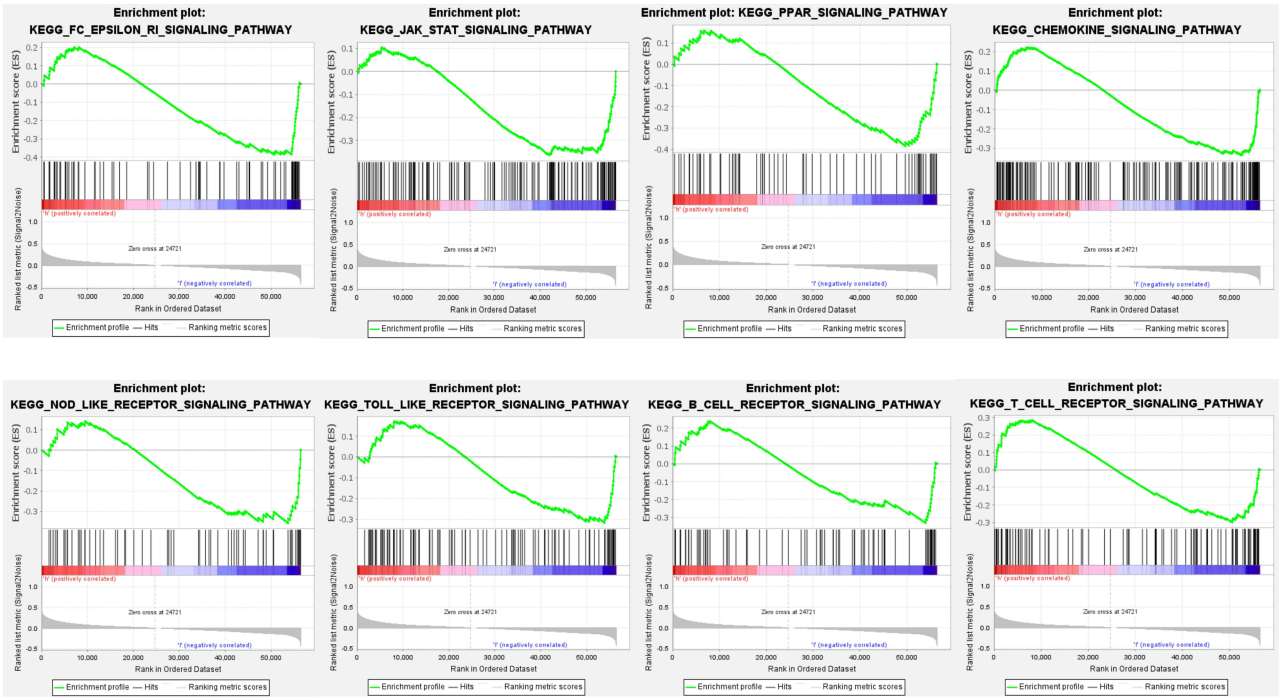


Figure 3 GSEA of the signaling pathways involved in the regulation of HDAC1 in NSCLC.

Overexpression of HDAC1 Mediates the TME Immunosuppressive State in NSCLC

We performed a multidimensional enrichment analysis to investigate whether *HDAC1* mediates TME immunosuppression in NSCLC. Heatmap of enrichment analysis indicated that *HDAC1* downregulated the activation of the immune regulatory signaling pathways in the high-expression cohort compared to that in the low-expression cohort. These down-regulated immune signaling pathways include interferon alpha response signaling, interferon gamma response signaling, allograft rejection signaling, interleukin (IL) 6 JAK STAT3 signaling, inflammatory response signaling, complement response signaling, IL2 STAT5 signaling, and tumor necrosis factor (TNF)-α via nuclear factor kappa B signaling (Figure 4A). The ssGSEA demonstrated that the *HDAC1* high-expression cohort was significantly less enriched compared to the *HDAC1* low-expression cohort in these immune signaling pathways (Figure 4B; P-value < 0.05). Subsequently, we further analyzed the effect of *HDAC1* on immune cells in TME. Immune cell and functional enrichment analysis revealed that the *HDAC1* high-expression cohort was significantly less enriched than the low-expression cohort (Figure 4C; P-value < 0.05). The TISIDB analysis showed that most lymphocyte infiltration was significantly negatively correlated with *HDAC1* expression in LUAD and LUSC (Supplementary Figures 1 and 2). Additionally, the ESTIMATE algorithm was used to calculate the immune cell component in the overall *HDAC1* high- and low-expression cohorts. The results revealed that the immune cell component in the *HDAC1* high-expression cohort was significantly lower than those in the *HDAC1* low-expression cohort (Figure 4D). These findings provide additional evidence that *HDAC1* overexpression in NSCLC mediates the TME immunosuppressive state.

Overexpression of HDAC1 Mediates TME Alterations in NSCLC Consistent with the "Immune Desert" Phenotype

According to the study by Hegde et al, the TME can be classified into three phenotypes: "immune desert", "immune inflammation", and "immune evasion".[12] By reviewing the PubMed literature, we conclude that the "immune desert" phenotype has the following characteristics:

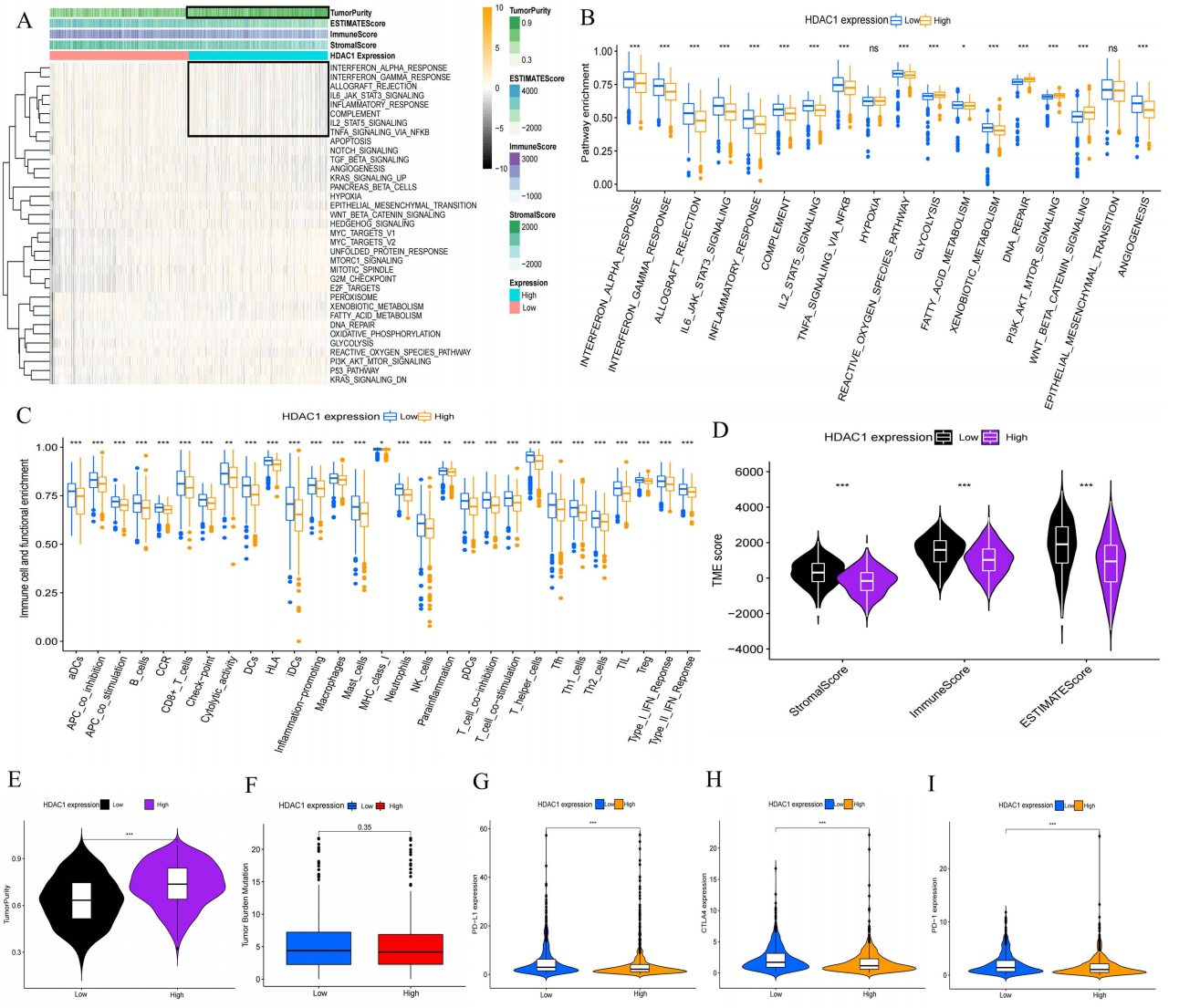


Figure 4 Analysis of the role of HDAC1 in TME of NSCLC. (**A**) Hallmark pathway gene set enrichment analysis in HDAC1 high- and low-expression groups. (**B**) Differential analysis of immunomodulatory signaling pathways enrichment in HDAC1 high- and low-expression groups. (**C**) Differential analysis of the enrichment of immune cells and functional gene sets in HDAC1 high- and low-expression groups. (**D** and **E**) ESTIMATE assessment of immune cell component, stromal component, and tumor component in HDAC1 high- and low-expression groups. (**F**) Differential analysis of tumor mutation burden in high and low HDAC1 expression groups. Differential analysis of (**G**) PD-L1, (**H**) PD-1, and (**I**) CTLA4 expression in HDAC1 high- and low-expression groups. *P-value < 0.05; **P-value < 0.01; ***P-value < 0.001; ns: not significant.

1. Little immune cell infiltration in the tumor parenchyma and interstitium.[12,22–24] As previously described, we observed significantly less immune cell infiltration in the *HDAC1* high-expression cohort than in the *HDAC1* low-expressing cohort (Figure 4C, Supplementary Figures 1 and 2). Although various types of immune cells are infiltrated by tumors, they can be broadly divided into two categories: those that actively kill tumors, such as natural killer (NK), NKT, and $CD8^+$ T cells; and those that actively suppress the immune cells responsible for killing tumors, such as regulatory T cells (Treg), type 1 helper T (Th1) and Th2 cells, and macrophages (M)1 and M2 cells.[25,26] However, our results demonstrated that the cellular levels of NK, NKT, $CD8^+$ T, Treg, Th1, Th2, M1, and M2 were all significantly reduced in the *HDAC1* high-expression cohort (Figure 4C, Supplementary Figures 1 and 2). Therefore, we hypothesized that *HDAC1* may be involved in NSCLC immunosuppression by suppressing NK, NKT, and $CD8^+$ T cells.
2. Lack of antigen presentation (low major histocompatibility complex (MHC) I expression).[12,24] As illustrated in Figure 4C, the MHC I enrichment in the *HDAC1* high-expression cohort was significantly lower compared to the *HDAC1* low-expression cohort.

https://doi.org/10.2147/JIR.S509316

3. Highly proliferative tumor.[12,24] Using the ESTIMATE algorithm, we calculated the tumor components in *HDAC1* low- and high-expression cohorts, and the results indicated that the tumor purity components in the *HDAC1* high-expression cohort were significantly more than those in the *HDAC1* low-expression cohort (Figure 4E). This implies that tumor proliferation was more evident in the *HDAC1* high-expression cohort.
4. Genome tends to be stable [low degree of tumor mutation burden (TMB)].[12,24] When compared to the *HDAC1* low-expression cohort, the DNA repair signaling was significantly highly enriched in the *HDAC1* high-expression cohort (Figure 4B). Additionally, TMB was estimated using TCGA dataset for the *HDAC1* high- and low-expression cohorts and the results suggested that the median TMB values in the *HDAC1* high-expression cohort were lower than those in the *HDAC1* low-expression cohort (Figure 4F).
5. Poor response to single agent ICIs. Immune checkpoints are frequently employed as a crucial indicator in clinical practice to evaluate the effectiveness of immunotherapy in patients.[27] We compared the expression levels of *PD-1, PD-L1*, and *CTLA4* using TCGA dataset to evaluate the impact of immunotherapy in *HDAC1* high- and low-expression cohorts. The findings demonstrated that the expression levels of *PD-1, PD-L1*, and *CTLA4* were significantly lower in the *HDAC1* high-expression cohort than those in the *HDAC1* low-expression cohort. This indicated that the effect of ICI monotherapy was lower in the *HDAC1* high-expression cohort than in the *HDAC1* low-expression cohort (Figure 4G–I; P-value < 0.05).

There are other atypical characteristics of "immune deserts". Rapidly proliferating tumor cells alter the metabolism of TME. The immune metabolism of T cells is altered by hypoxia, lactate production, the presence of acidic TME, and increased lipogenesis, which affects T cell activation, differentiation, and proliferation.[28–32] In contrast to the *HDAC1* low-expression cohort, we observed that the *HDAC1* high-expression cohort was highly enriched in hypoxic and glycolytic signaling. However, the *HDAC1* high-expression cohort was significantly less enriched in reactive oxygen species signaling. These findings suggest that high expression of *HDAC1* mediates a hypoxic, acidic microenvironment in NSCLC. Meanwhile, in the *HDAC1* high-expression cohort fatty acid metabolism and xenobiotic metabolic signaling were significantly downregulated compared to those in the *HDAC1* low-expression cohort, which also suggested that high expression of *HDAC1* causes increased adipogenesis in NSCLC (Figure 4A). Therefore, high expression of *HDAC1* causes TME alterations in NSCLC that prevent T cell activation and proliferation.

Studies have demonstrated that some classical signaling pathways are also closely related to TME immunosuppression. Early T cell initiation and infiltration can be affected by the WNT β-catenin signaling pathway, which reduces the gradient of chemokines CXCL1, CXCL2, and CCL4 required for CD103 dendritic cells (DCs).[33] Phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) signaling can reprogram tumor cell metabolism to suppress T cell function through regulation.[34] Consistently, our study showed that the *HDAC1* high-expression cohort was highly enriched in WNT β-catenin and PI3K AKT signaling (Figure 4A and B).

Conclusively, we observed that *HDAC1* overexpression in NSCLC mediates the "immune desert" phenotype of TME.

Immune Cell Distribution Demonstrates That HDAC1 Overexpression in NSCLC Mediates Immunosuppressive TME Formation

The core of the "immune desert", "immune evasion", and "immune inflammation" classification by Hegde et al, is based on the distribution of $CD8^+$ T cells in the tumor stroma and parenchyma.[12] To demonstrate the aforementioned bioinformatics findings, we performed $CD8^+$ T cell staining using the immunohistochemical staining technique on samples with high *HDAC1* expression in NSCLC. We observed slight infiltration of $CD8^+$ T cells in the tumor tissue and stroma of *HDAC1*-overexpressing samples, which was consistent with the "immune desert" phenotype (Figure 5A–F). Moreover, the amount of CD8+ T-cell infiltration was significantly increased in NSCLC samples with low HDAC1 expression compared to those with high HDAC1 expression (Figure 5G–L). Therefore, we concluded that *HDAC1* overexpression in NSCLC mediates the formation of TME immunosuppression.

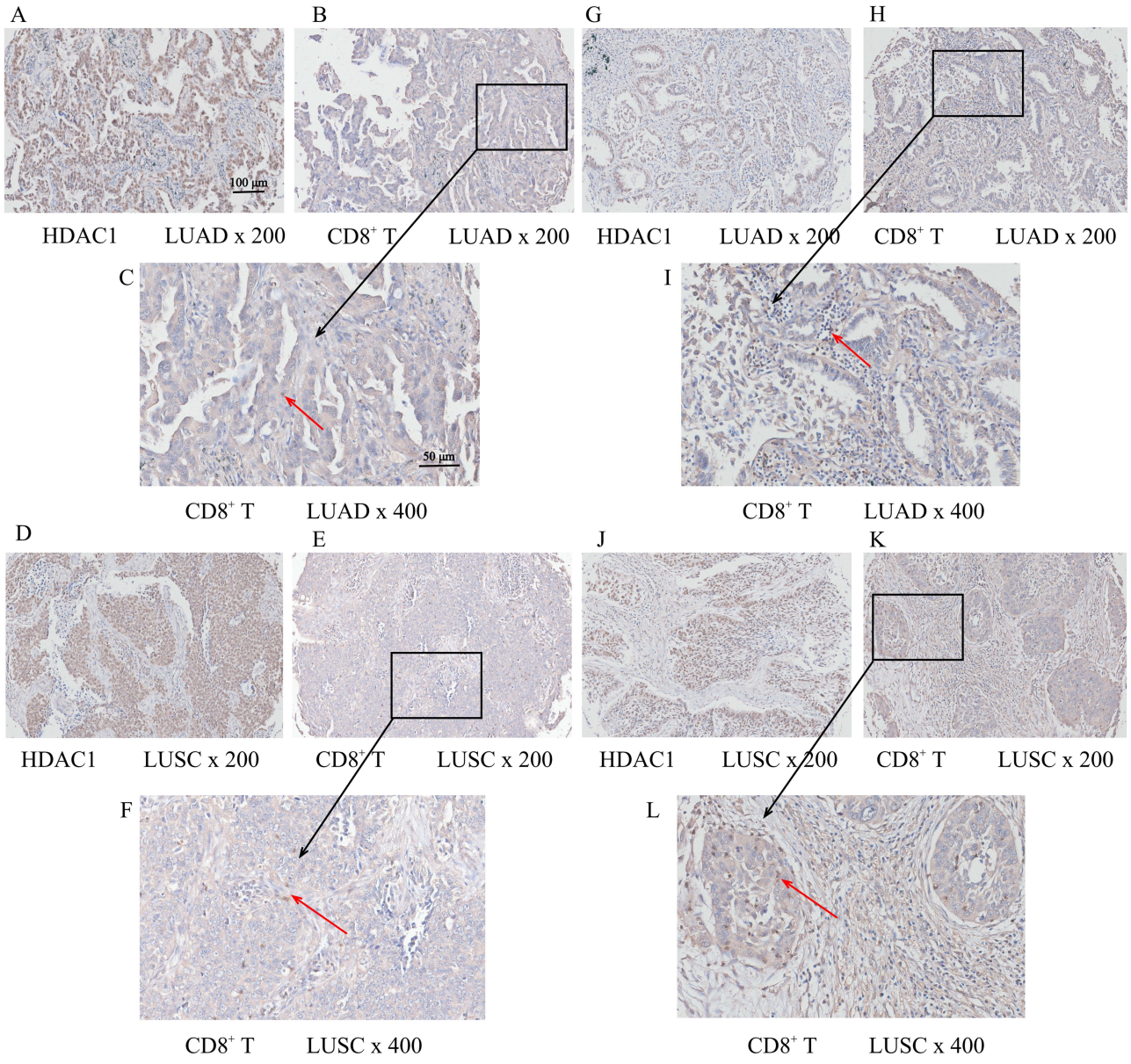


Figure 5 Distribution of $CD8^+$ T cells in HDAC1 high-expression and low-expression tissue samples by immunohistochemical staining. Distribution of $CD8^+$ T cells in HDAC1 high-expression samples in (**A–C**) LUAD and (**D–F**) LUSC tissue samples. Distribution of $CD8^+$ T cells in HDAC1 low-expression samples in (**G–I**) LUAD and (**J–L**) LUSC tissue samples. Red arrow: $CD8^+$ T.

Discussion

TME is a complex integrated system on which tumor cells depend for survival. Studies have demonstrated that TME has multiple effects on T cells. For example, TME can modulate the infiltration of T cells from the circulatory system into the tumor interior by post-translationally altering the ability of chemokines and overriding chemotactic signals that recruit T cells to the tumor.[10,35,36] Products of bone marrow monocytes such as Fas ligand, TNF-α and TNF-related apoptosis inducing ligand in TME can also induce apoptosis and thus affect T-cell survival.[10] In addition, TME not only alters T cell replication by affecting $CD103^+$, Baft3-dependent (dendritic cells) DCs, but also indole 2,3-dioxygenase expressed by DCs.[37,38] Myeloid-derived suppressor cells and cancer cells in TME can catabolize tryptophan and produce uranine, thereby impairing T cell proliferation in tumors.[39–41] Studies have demonstrated that oncogenes and abnormal pathway signaling are at the core of this immunosuppressive TME established in tumor patients.[42] Therefore, it is important to find suitable genetic targets to alter immunosuppressive TME and improve patients' long-term survival.

https://doi.org/10.2147/JIR.S509316

In this study, we further explored the impact of *HDAC1* on immune regulation in TME of NSCLC based on the finding of Lawson et al that *HDAC1* enables cancer cells to evade killing mediated by cytotoxic T lymphocytes.[19] Pathway enrichment analysis indicated that *HDAC1* downregulated immune regulatory signaling pathways in NSCLC, while immune cell infiltration analysis revealed that *HDAC1* expression in NSCLC was significantly negatively correlated with immune cell infiltration. In addition, the TME characteristics of *HDAC1* high and low expression cohorts were compared by bioinformatics, and the results showed that the TME in the *HDAC1* high expression cohort was consistent with the "immune desert" phenotype. Finally, *HDAC1* overexpression in NSCLC was demonstrated to mediate the TME immunosuppressive state by immunohistochemical staining experiments on $CD8^+$ T cells. Therefore, our findings suggested that *HDAC1* may serve as a core target to alter immunosuppressive TME in NSCLC, leading to a new breakthrough in NSCLC immunotherapy.

HDAC1, as a key member of epigenetic regulation, plays a crucial role in chromatin remodeling and gene expression through histone deacetylation. It is extensively involved in tumor biology, particularly in tumor immune regulation, making it a promising therapeutic target for cancer treatment.[15] *HDAC1* regulates dendritic cell (DC) development by modulating the expression, chromatin accessibility, and histone acetylation of transcription factors IRF4, IRF8, and SPIB. This process promotes cDC2 differentiation while impairing pDC generation and enhancing cDC1 maturation, thereby influencing anti-tumor immune responses.[43] Additionally, *HDAC1* and HDAC2 are essential for T-cell development and genomic stability through their roles in chromatin structure modulation and TCR signaling pathways, with their dual deletion leading to developmental arrest, impaired TCR signaling, and tumorigenic transformation of immature T cells.[44] Previous studies further demonstrate *HDAC1*'s involvement in regulating the immune state across various tumor types. For example, *HDAC1* interacts with exosomal circ_0006896 to impair $CD8^+$ T-cell function by reducing LEF1 transcription and cytotoxic molecule expression, facilitating immune escape in acute myeloid leukemia.[45] Similarly, the CTBP1/*HDAC1*/HDAC2 complex suppresses MAT1A transcription, leading to decreased ferroptosis and reduced $CD8^+$ T-cell cytotoxicity, thereby promoting immune escape in hepatocellular carcinoma.[46] Moreover, *HDAC1* is implicated in immune therapy resistance. Overexpression of *HDAC1*/2/3 inhibits IFNγ signaling in hepatocellular carcinoma, contributing to immune checkpoint blockade resistance.[47] *HDAC1* also mediates immune therapy resistance by reducing T-cell infiltration and CTL sensitivity.[48] *HDAC1* upregulation suppresses cell cycle inhibitors and apoptotic regulators, fostering a stem-like, immune-resistant tumor phenotype and diminishing anti-tumor immune responses.[49] These findings underscore *HDAC1* as a central regulator of immune evasion and resistance, highlighting its potential as a therapeutic target in immuno-oncology.

However, there are some shortcomings in our study. For example, the results of this study were only briefly validated at the tissue sample level and were not further investigated at the cellular level. Multiple genetic alterations may be present in tissue samples. This study selected tissue samples with high *HDAC1* expression for immunohistochemical staining of $CD8^+$ T cells failing to exclude other genetic interference. In addition, the specific mechanism of *HDAC1*-mediated TME immunosuppression in NSCLC was not investigated in this study.

Collectively, our study suggests that HDAC1 overexpression in NSCLC mediates the TME immunosuppressive state.

Abbreviations

HDAC, histone deacetylase; NSCLC, non-small cell lung cancer; AOD, average optical density; CTLA4, cytotoxic T-lymphocyte-associated protein 4 receptor; GSVA, Gene Set Variation Analysis; ICIs, Immune checkpoint inhibitors; LUSC, lung squamous carcinoma; GSEA, gene set enrichment analysis; LUAD, lung adenocarcinoma; PD-L1, programmed death ligand 1; NK, natural killer; TCGA, the Cancer Genome Atlas; TMA, tissue microarray; PPAR, peroxisome proliferator-activated receptor; TME, tumor microenvironment; Treg, regulatory T cells; NOD, nucleotide-binding oligomerization domain; IL, interleukin; TMB, tumor mutation burden; TNF, tumor necrosis factor; Th1, type 1 helper; M, macrophages; MHC, histocompatibility complex; TMB, tumor mutation burden; PI3K, Phosphatidylinositol 3-kinase; PD-1, programmed cell death protein 1; ssGSEA, single-sample Gene Set Enrichment Analysis; AKT, protein kinase B; DCs, dendritic cells; ESTIMATE, estimation of stromal and immune cells in malignant tumor tissues using expression data; DC, dendritic cell.

Data Sharing Statement

The raw data used in this study were downloaded from TCGA database: https://www.cancer.gov/, TISIDB database: http://cis.hku.hk/TISIDB/index.php, Molecular Signatures Database: http://www.gsea-msigdb.org/gsea/login.jsp and Kaplan Meier plotter database: https://kmplot.com/analysis/.

Ethics Statement and Informed Consent

The study involving human participants was conducted in accordance with the principles outlined in the Declaration of Helsinki. Ethical approval was granted by the Research Ethics Committee of the Second People's Hospital of Changzhou and the Third Affiliated Hospital of Nanjing Medical University ([2023]KY221-01). Prior to participation, all individuals provided written informed consent after being thoroughly informed about the study's objectives, procedures, potential risks, and benefits, as well as their right to withdraw at any point without prejudice.

Acknowledgments

We thank the FigDraw platform for the scientific drawing capabilities and Bullet Edits Limited for language editing and proofreading of the manuscript.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This study is supported by the Major Science and Technology Projects of the Changzhou Science and Technology Bureau (Grant number: CE20205047).

Disclosure

The authors declare that there is no conflict of interest in this study.

References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA Cancer J Clin*. 2022;72(1):7–33. doi:10.3322/caac.21708
2. Molina JR, Yang P, Cassivi SD, Schild SE, Adjei AA. Non-small cell lung cancer: epidemiology, risk factors, treatment, and survivorship. *Mayo Clin Proc*. 2008;83(5):584–594. doi:10.4065/83.5.584
3. Herbst RS, Morgensztern D, Boshoff C. The biology and management of non-small cell lung cancer. *Nature*. 2018;553(7689):446–454. doi:10.1038/nature25183
4. Thai AA, Solomon BJ, Sequist LV, Gainor JF, Heist RS. Lung cancer. *Lancet*. 2021;398(10299):535–554. doi:10.1016/s0140-6736(21)00312-3
5. Zhang Y, Zhang Z. The history and advances in cancer immunotherapy: understanding the characteristics of tumor-infiltrating immune cells and their therapeutic implications. *Cell mol Immunol*. 2020;17(8):807–821. doi:10.1038/s41423-020-0488-6
6. Marabelle A, Tselikas L, de Baere T, Houot R. Intratumoral immunotherapy: using the tumor as the remedy. *Ann Oncol*. 2017;28(suppl_12):xii33–xii43. doi:10.1093/annonc/mdx683
7. Reck M, Remon J, Hellmann MD. First-line immunotherapy for non-small-cell lung cancer. *J Clin Oncol*. 2022;40(6):586–597. doi:10.1200/jco.21.01497
8. Reckamp KL, Redman MW, Dragnev KH, et al. Phase II randomized study of ramucirumab and pembrolizumab versus standard of care in advanced non-small-cell lung cancer previously treated with immunotherapy-lung-MAP S1800A. *J Clin Oncol*. 2022;40(21):2295–2306. doi:10.1200/jco.22.00912
9. Hinshaw DC, Shevde LA. The tumor microenvironment innately modulates cancer progression. *Cancer Res*. 2019;79(18):4557–4566. doi:10.1158/0008-5472.Can-18-3962
10. Pitt JM, Marabelle A, Eggermont A, Soria JC, Kroemer G, Zitvogel L. Targeting the tumor microenvironment: removing obstruction to anticancer immune responses and immunotherapy. *Ann Oncol*. 2016;27(8):1482–1492. doi:10.1093/annonc/mdw168
11. Xiao Y, Yu D. Tumor microenvironment as a therapeutic target in cancer. *Pharmacol Ther*. 2021;221:107753. doi:10.1016/j.pharmthera.2020.107753
12. Hegde PS, Karanikas V, Evers S. The where, the when, and the how of immune monitoring for cancer immunotherapies in the era of checkpoint inhibition. *Clin Cancer Res*. 2016;22(8):1865–1874. doi:10.1158/1078-0432.Ccr-15-1507
13. Fan Y, Zhou Y, Lou M, Li X, Zhu X, Yuan K. m(6)A regulator-mediated methylation modification patterns and characterisation of tumour microenvironment infiltration in non-small cell lung cancer. *J Inflamm Res*. 2022;15:1969–1989. doi:10.2147/jir.S356841

14. Bader JE, Voss K, Rathmell JC. Targeting metabolism to improve the tumor microenvironment for cancer immunotherapy. *Mol Cell*. 2020;78(6):1019–1033. doi:10.1016/j.molcel.2020.05.034
15. Dunaway LS, Pollock JS. HDAC1: an environmental sensor regulating endothelial function. *Cardiovasc Res*. 2022;118(8):1885–1903. doi:10.1093/cvr/cvab198
16. King J, Patel M, Chandrasekaran S. Metabolism, HDACs, and HDAC inhibitors: a systems biology perspective. *Metabolites*. 2021;11(11):792. doi:10.3390/metabo11110792
17. Zhang L, Bu L, Hu J, et al. HDAC1 knockdown inhibits invasion and induces apoptosis in non-small cell lung cancer cells. *Biol Chem*. 2018;399(6):603–610. doi:10.1515/hsz-2017-0306
18. Lin CW, Wang LK, Wang SP, et al. Daxx inhibits hypoxia-induced lung cancer cell metastasis by suppressing the HIF-1α/HDAC1/Slug axis. *Nat Commun*. 2016;7(1):13867. doi:10.1038/ncomms13867
19. Lawson KA, Sousa CM, Zhang X, et al. Functional genomic landscape of cancer-intrinsic evasion of killing by T cells. *Nature*. 2020;586(7827):120–126. doi:10.1038/s41586-020-2746-2
20. Iasonos A, Schrag D, Raj GV, Panageas KS. How to build and interpret a nomogram for cancer prognosis. *J Clin Oncol*. 2008;26(8):1364–1370. doi:10.1200/jco.2007.12.9791
21. Liberzon A, Birger C, Thorvaldsdóttir H, Ghandi M, Mesirov JP, Tamayo P. The molecular signatures database (MSigDB) hallmark gene set collection. *Cell Syst*. 2015;1(6):417–425. doi:10.1016/j.cels.2015.12.004
22. Alexandrov LB, Nik-Zainal S, Wedge DC, et al. Signatures of mutational processes in human cancer. *Nature*. 2013;500(7463):415–421. doi:10.1038/nature12477
23. Kandoth C, McLellan MD, Vandin F, et al. Mutational landscape and significance across 12 major cancer types. *Nature*. 2013;502(7471):333–339. doi:10.1038/nature12634
24. Hegde PS, Chen DS. Top 10 challenges in cancer immunotherapy. *Immunity*. 2020;52(1):17–35. doi:10.1016/j.immuni.2019.12.011
25. Nagarsheth N, Wicha MS, Zou W. Chemokines in the cancer microenvironment and their relevance in cancer immunotherapy. *Nat Rev Immunol*. 2017;17(9):559–572. doi:10.1038/nri.2017.49
26. Desbois M, Udyavar AR, Ryner L, et al. Integrated digital pathology and transcriptome analysis identifies molecular mediators of T-cell exclusion in ovarian cancer. *Nat Commun*. 2020;11(1):5583. doi:10.1038/s41467-020-19408-2
27. Wu M, Huang Q, Xie Y, et al. Improvement of the anticancer efficacy of PD-1/PD-L1 blockade via combination therapy and PD-L1 regulation. *J Hematol Oncol*. 2022;15(1):24. doi:10.1186/s13045-022-01242-2
28. Semenza GL. Intratumoral hypoxia and mechanisms of immune evasion mediated by hypoxia-inducible factors. *Physiology*. 2021;36(2):73–83. doi:10.1152/physiol.00034.2020
29. Certo M, Tsai CH, Pucino V, Ho PC, Mauro C. Lactate modulation of immune responses in inflammatory versus tumour microenvironments. *Nat Rev Immunol*. 2021;21(3):151–161. doi:10.1038/s41577-020-0406-2
30. Renner K, Singer K, Koehl GE, et al. Metabolic hallmarks of tumor and immune cells in the tumor microenvironment. *Front Immunol*. 2017;8:248. doi:10.3389/fimmu.2017.00248
31. Wang JX, Choi SYC, Niu X, et al. Lactic acid and an acidic tumor microenvironment suppress anticancer immunity. *Int J mol Sci*. 2020;21(21):8363. doi:10.3390/ijms21218363
32. Luo Y, Wang H, Liu B, Wei J. Fatty acid metabolism and cancer immunotherapy. *Curr Oncol Rep*. 2022;24(5):659–670. doi:10.1007/s11912-022-01223-1
33. Mariathasan S, Turley SJ, Nickles D, et al. TGFβ attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells. *Nature*. 2018;554(7693):544–548. doi:10.1038/nature25501
34. O'Donnell JS, Massi D, Teng MWL, Mandala M. PI3K-AKT-mTOR inhibition in cancer immunotherapy, redux. *Semin Cancer Biol*. 2018;48:91–103. doi:10.1016/j.semcancer.2017.04.015
35. Molon B, Ugel S, Del Pozzo F, et al. Chemokine nitration prevents intratumoral infiltration of antigen-specific T cells. *J Exp Med*. 2011;208(10):1949–1962. doi:10.1084/jem.20101956
36. Motz GT, Santoro SP, Wang LP, et al. Tumor endothelium FasL establishes a selective immune barrier promoting tolerance in tumors. *Nat Med*. 2014;20(6):607–615. doi:10.1038/nm.3541
37. Broz ML, Binnewies M, Boldajipour B, et al. Dissecting the tumor myeloid compartment reveals rare activating antigen-presenting cells critical for T cell immunity. *Cancer Cell*. 2014;26(5):638–652. doi:10.1016/j.ccell.2014.09.007
38. Hildner K, Edelson BT, Purtha WE, et al. Batf3 deficiency reveals a critical role for CD8alpha+ dendritic cells in cytotoxic T cell immunity. *Science*. 2008;322(5904):1097–1100. doi:10.1126/science.1164206
39. Platten M, Wick W, Van den Eynde BJ. Tryptophan catabolism in cancer: beyond IDO and tryptophan depletion. *Cancer Res*. 2012;72(21):5435–5440. doi:10.1158/0008-5472.Can-12-0569
40. Munn DH, Mellor AL. Indoleamine 2,3 dioxygenase and metabolic control of immune responses. *Trends Immunol*. 2013;34(3):137–143. doi:10.1016/j.it.2012.10.001
41. Joyce JA, Fearon DT. T cell exclusion, immune privilege, and the tumor microenvironment. *Science*. 2015;348(6230):74–80. doi:10.1126/science.aaa6204
42. Binnewies M, Roberts EW, Kersten K, et al. Understanding the tumor immune microenvironment (TIME) for effective therapy. *Nat Med*. 2018;24(5):541–550. doi:10.1038/s41591-018-0014-x
43. De Sá Fernandes C, Novoszel P, Gastaldi T, et al. The histone deacetylase HDAC1 controls dendritic cell development and anti-tumor immunity. *Cell Rep*. 2024;43(6):114308. doi:10.1016/j.celrep.2024.114308
44. Dovey OM, Foster CT, Conte N, et al. Histone deacetylase 1 and 2 are essential for normal T-cell development and genomic stability in mice. *Blood*. 2013;121(8):1335–1344. doi:10.1182/blood-2012-07-441949
45. Can C, Yang X, Jia H, et al. Exosomal circ_0006896 promotes AML progression via interaction with HDAC1 and restriction of antitumor immunity. *mol Cancer*. 2025;24(1):4. doi:10.1186/s12943-024-02203-8
46. Li Y, Hu G, Huang F, et al. MAT1A Suppression by the CTBP1/HDAC1/HDAC2 transcriptional complex induces immune escape and reduces ferroptosis in hepatocellular carcinoma. *Lab Invest*. 2023;103(8):100180. doi:10.1016/j.labinv.2023.100180

47. Tu Y, Wu H, Zhong C, et al. Pharmacological activation of STAT1-GSDME pyroptotic circuitry reinforces epigenetic immunotherapy for hepatocellular carcinoma. *Gut*. 2024:gutjnl–2024–332281. doi:10.1136/gutjnl-2024-332281
48. Oh SJ, Lee HJ, Song KH, et al. Targeting the NANOG/HDAC1 axis reverses resistance to PD-1 blockade by reinvigorating the antitumor immunity cycle. *J Clin Invest*. 2022;132(6). doi:10.1172/jci147908
49. Song KH, Choi CH, Lee HJ, et al. HDAC1 upregulation by NANOG promotes multidrug resistance and a stem-like phenotype in immune edited tumor cells. *Cancer Res*. 2017;77(18):5039–5053. doi:10.1158/0008-5472.Can-17-0072