High *HDAC5* expression correlates with a poor prognosis and the tumor immune microenvironment in gastric cancer

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Background: Gastric cancer (GC) is one of the most common malignant tumors worldwide and has a poor prognosis. Previous studies have confirmed differential histone deacetylase 5 (*HDAC5*) expression in various common tumors. *HDAC5* is also associated with prognosis and plays a role in cancer cell proliferation, invasion, and metastasis, as well as the tumor immune microenvironment (TIME). However, *HDAC5* in GC is not well understood. The aims of study were to investigate the *HDAC5* expression correlates with prognosis and the TIME in GC.

Methods: A total of 355 tumor tissues and 300 matched paracancerous tissues were collected from GC patients who underwent radical surgery. The correlation between clinicopathological characteristics, immune-related factors and *HDAC5* expression were analyzed. Univariate and multivariate Cox regression analyses were used to confirm the independent factors affecting the prognosis of GC. Survival curves were plotted using the Kaplan-Meier method. Furthermore, the stomach adenocarcinoma (STAD) dataset was downloaded from The Cancer Genome Atlas (TCGA). The expression levels of *HDAC5* were defined as high or low using the gene set variance analysis (GSVA) package. Identification of differential immune infiltrating cells was performed by single sample gene set enrichment analysis (ssGSEA).

Results: The positive expression rate of *HDAC5* was higher in tumor tissues than in paracancerous tissues (38.87% *vs.* 14.67%, P<0.001). Univariate and multivariate Cox analyses showed that *HDAC5* was an independent factor affecting the prognosis of GC. The *HDAC5* expression levels were correlated with age (P=0.046), smoking history (P=0.001), Lauren type (P=0.042), and pM stage (P=0.012). Furthermore, these levels were correlated with $CD3^+$ T cells (P<0.001), $CD4^+$ T cells (P<0.001), $CD8^+$ T cells (P<0.001) and PD-L1 (P=0.001). Further analysis of patients in TCGA cohort confirmed the association between *HDAC5* and activated CD4 T cells, activated CD8 T cells, and other immune infiltrating cells.

Conclusions: *HDAC5* is highly expressed in tumor tissues and is an independent factor affecting the prognosis of GC. Additionally, *HDAC5* can regulate the TIME of GC and is a potential target for immunotherapy.

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Keywords: Gastric cancer (GC); histone deacetylase 5 (*HDAC5*); prognosis; tumor immune microenvironment (TIME); tumor-infiltrating lymphocytes (TILs)

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Introduction

Gastric cancer (GC) is a global health problem, with more than one million new diagnoses worldwide every year. Although incidence and mortality rates have declined over the past 5 years, the latest statistics report that GC ranks fifth in terms of incidence and fourth in terms of mortality among all malignancies (1). Early diagnosis of GC is difficult; currently, the early diagnosis of GC mainly relies on imaging, serum tumor markers, endoscopy, and biopsy pathology (2), which are limited by the cumbersome process or insufficient specificity. Surgery is still the main treatment for GC, but with the combination of chemotherapy, radiotherapy, and targeted therapy, the prognosis of GC has improved significantly (3). However, the clinical efficacy of conventional therapy is limited and the prognosis remains relatively poor. As a recent breakthrough, immunotherapy has become an effective treatment modality after surgery, chemotherapy, radiotherapy, and targeted therapy (4). Thus, there is a pressing need to identify new specific markers and potential targets related to immunotherapy to improve the diagnosis and treatment of GC.

First identified in the mouse genome in 1999, histone deacetylase 5 (*HDAC5*) is a member of the *HDAC* class IIa family (5). This protein consists of 1,122 amino acids, has a molecular weight of 121.9 kDa, and has C-terminal deacetylase and N-terminal adapter domains. *HDAC5* is known to be expressed in the lung, brain, myocardium, skeletal muscle, and placenta, and many studies have shown that *HDAC5* is differentially expressed in different types of tumors. Previous research has confirmed that *HDAC5* expression is upregulated in breast cancer (BC) (6), hepatocellular carcinoma (HCC) (7), lung cancer (LC) (8), pancreatic neuroendocrine tumors (pNETs) (9), and colorectal cancer (CRC) (10). It has also been shown that *HDAC5* affects cancer cell proliferation, invasion, apoptosis, and cell cycle progression. Zhong *et al.* demonstrated that overexpression of *HDAC5* significantly promotes tumor cell proliferation and invasion and inhibits apoptosis by constructing LC cell lines; meanwhile, knockdown of *HDAC5* significantly inhibits tumor cell proliferation and invasion and promotes apoptosis (8). He *et al.* found that *HDAC5* messenger RNA (mRNA) and protein levels were upregulated in human CRC cell lines, and the cell counting kit-8 (CCK-8) assay showed that overexpression of *HDAC5* promotes the proliferation of CRC cells. However, knockdown of *HDAC5* was observed to inhibit the growth of CRC cells (11). In addition, a study by Peixoto *et al.* showed that *HDAC5* was associated with the active replication of perisynaptic heterochromatin in the late S phase, and the specific depletion of *HDAC5* by RNA interference led to structural changes in heterochromatin. This defect in heterochromatin maintenance and assembly was sensed by the DNA damage checkpoint pathway, triggering autophagy and apoptosis in cancer cells (12).

Immune checkpoint inhibitors (ICIs), such as anti-programmed cell death-1 (PD-1) or programmed cell death ligand-1 (PD-L1) monoclonal antibodies, are the new standard of targeted therapy for advanced or metastatic GC and have shown some prognostic improvement in clinical trials (13,14). The tumor immune microenvironment (TIME) is the internal environment of malignant tumor progression and site of the host antitumor immune response and normal tissue destruction. Tumor-infiltrating lymphocytes (TILs) are an important part of the TIME; TILs include $CD3^+$ T cells, $CD4^+$ T cells, and $CD8^+$ T cells, which can reflect the host antitumor immune response (15). *HDACs* are associated with the immune response, and *HDAC5* interacts with the immune system (including immune cells and inflammatory cytokines) during cancer development and progression. *HDAC5* is associated with macrophage differentiation in lymphoma cells (16), and depletion of *HDAC5* in lymphoma cells via stimulation of nuclear factor-κB (NF-κB) activity reduces the levels of tumor necrosis factor-α (TNF-α) and monocyte chemotactic protein-1 (MCP-1) (17), suggesting a regulatory function of *HDAC5* in the proinflammatory response of macrophages. In pancreatic cancer, Zhou *et al.* revealed an unknown role of *HDAC5* in regulating NF-κB signaling pathway and antitumor immune response (18). And in GC, Deng

et al. confirmed that HDAC is essential for interferon-γ (IFN-γ)-induced B7-H1 in GC, and suggests the possibility of targeting B7-H1 using small molecular HDAC inhibitors for cancer treatment (19). Hence, HDAC has a role in immunotherapy and the value of *HDAC5* in the immune microenvironment of GC needs to be explored.

In this study, we assessed the expression level of *HDAC5* in 355 tumor tissues and 300 paracancerous tissues by immunohistochemistry (IHC). Independent factors affecting the prognosis of GC were analyzed by univariate and multivariate analyses. The expression levels of $CD3^+$ T cell, $CD4^+$ T cell, and $CD8^+$ T cell markers and PD-L1 were also measured to compare the correlations between *HDAC5* and PD-L1 *vs. HDAC5* and TILs. Further analysis of GC samples in The Cancer Genome Atlas (TCGA) was performed to identify differential immune infiltrating cells and jointly investigate the role of *HDAC5* in the immune microenvironment of GC. We present the following article in accordance with the REMARK reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-4325/rc).

Methods

Patients

This study enrolled 355 patients who were admitted to The Cancer Hospital of the University of Chinese Academy of Sciences (Zhejiang Cancer Hospital) and underwent radical surgery for GC between July 2008 and July 2017. The inclusion criteria were as follows: (I) pathological diagnosis of GC; (II) relatively complete medical records; (III) no preoperative integrated antitumor therapy, such as radiotherapy, targeted therapy, or immunotherapy; and (IV) complete survival follow-up data. The exclusion criteria were as follows: (I) other types of malignant tumors; (II) metastasis from other malignant tumors; and (III) severe cardiopulmonary insufficiency, renal insufficiency, and other underlying diseases.

We collected the data from the inpatient medical records system, including demographic characteristics and clinicopathological features. The pathological stage was determined according to the American Joint Committee on Cancer (AJCC) 8^{th} edition system. Survival information was obtained by telephone follow-up and medical records, and the last follow-up visit was conducted in August 2021. Overall survival (OS) was defined as the duration from the initial surgery to death or the last follow-up visit. In addition, a dataset containing 375 stomach adenocarcinoma (STAD) tumor tissue samples was downloaded from TCGA.

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of Cancer Hospital of the University of Chinese Academy of Sciences (Zhejiang Cancer Hospital) (No. IRB-2021-431) and informed consent was taken from all the patients

IHC

The 355 GC tumor tissues and 300 paracancerous tissues were collected, fixed in formalin, and embedded in paraffin. Two veteran pathologists independently selected representative tissues for tissue microarrays (TMAs). The sections were dewaxed separately and rinsed with distilled water; then, antigen repair was performed by washing with phosphate-buffered saline (PBS) for 5 min (three times). Next, the primary antibody (HDAC5: 16166-1-AP; CD3: ab16669; CD4: ab133616; CD8: ab17147; PD-L1: SK006) was added, incubated overnight at 4 ℃, and washed with PBS for 5 min (three times). Subsequently, goat anti-rabbit immunoglobulin G (IgG) H&L (SP-9000, ZSGB-BIO Corp., Shanghai, China) was added to the TMAs (dilution ratio 1:1,000), incubated for 30 min, and washed with PBS for 5 min (three times). 3,3'-diaminobenzidine (DAB) color development and hematoxylin restraining of cell nuclei were then performed using a DAB color development kit. Finally, the TMAs were dehydrated and closed with neutral gel.

IHC assessment

IHC staining of *HDAC5* was interpreted separately by two pathologists using the H-score system. The formula for the H-score system was as follows: H score = (∑IS × AP), where IS indicates the staining intensity and AP indicates the percentage of positively stained cells. IS was determined by the cell staining: 0 for no staining; 1 for weak staining; 2 for moderate staining; and 3 for strong staining. AP was recorded as follows: 0 for 0% stained cells; 1 for 1–25% stained cells; 2 for 26–50% stained cells; 3 for 51–75% stained cells; and 4 for 76–100% stained cells. A H-score =6 was set as the cutoff value, and the patients were divided into groups according to *HDAC5* expression (positive *vs.* negative).

PD-L1 expression was recorded based on the combined positivity score (CPS) score, CPS = [number of PD-L1-

positive cells (tumor cells, lymphocytes, macrophages)/ total tumor cells] × 100 for evaluation, where a CPS ≥10 was considered positive. TILs were quantified by pathologists who observed and recorded the total number of corresponding lymphocytes in the entire magnification field and divided the samples into high and low expression groups using the median as the cutoff value.

Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp, Armonk, NY, USA) and GraphPad Prism for Windows, version 8.3.0 (GraphPad Software, San Diego, CA, USA). Counting data were expressed as frequencies and percentages, and measurement data were expressed as $\bar{x}\pm s$. The correlation between *HDAC5* expression levels and the clinicopathological features and immune-related factors was determined by the Mann-Whitney test or chi-square test. Survival curves were plotted by the Kaplan-Meier method, and independent factors affecting the prognosis of patients with GC were determined by univariate and multivariate Cox regression analyses. Factors in multivariate analyses were selected according to the importance of clinical information. The hazard ratios (HRs) and their corresponding 95% confidence intervals (CIs) were also calculated. TCGA data were analyzed by single sample gene set enrichment analysis (ssGSEA) using the gene set variance analysis (GSVA) package to identify differentially infiltrated immune cells (Wilcoxon rank-sum test), and heat maps and violin plots were generated according to the gene expression level of *HDAC5* (high *vs.* low). P<0.05 was considered to indicate a statistically significant difference.

Results

Clinicopathological features of the 355 GC patients

The mean age of the 355 GC patients included in the study was 63.74 years, with a median age of 64 years and an age range of 28–91 years. Of these patients, 256 (72.11%) were men, while only 99 (27.89%) were women. Adenocarcinoma was the main pathological type among the included patients (n=322, 90.70%), and most tumors were undifferentiated or hypodifferentiated (47.89% collectively). In terms of the tumor site, most tumors were considered distal GC (226 cases, 63.66%), and only 116 cases (32.68%) were proximal GC. According to the pTNM stage, most of the assessed tumors were stage III (72.68%), with stages I, II, and III accounting for 4.79%, 14.37%, and 6.20% of tumors, respectively. Detailed clinicopathological information is shown in *Table 1*.

HDAC5 is highly expressed in GC tissues and predicts a poor prognosis

We found that *HDAC5* was expressed in both the cytoplasm and nucleus by IHC staining. Representative immunohistochemical plots (×200-fold) and specific H-scores are shown in *Figure 1A,1B*. Among the 355 tumor TMAs, 268 had different levels of *HDAC5* expression, and the *HDAC5* expression rate was 75.49%. Eighty-seven patients were negative for *HDAC5* expression, accounting for 24.51% (*Table 2*). In this study, we defined H-score =6 as the cutoff value, H-score ≥6 as the *HDAC5*-positive expression group, and H-score <6 as the *HDAC5*-negative expression group. The results showed that 138 of 355 GC tissues (38.87%) exhibited high *HDAC5* expression, while only 44 of 300 paracancerous tissues (14.67%) exhibited high *HDAC5* expression, which indicated that *HDAC5* was upregulated in GC tissues compared with the paracancerous tissues (P<0.001; *Table 3*).

The effect of *HDAC5* on prognosis has been confirmed in other types of tumors. To investigate its effect on the prognosis of GC, we used the Kaplan-Meier method to plot survival curves. The prognosis of patients with high *HDAC5* expression levels in tumor tissues was found to be worse than that of those with low *HDAC5* expression in tumor tissues (5-year OS: 44.7% *vs.* 60.6%, P=0.007; *Figure 1C*). However, there was no significant correlation in the paracancerous tissues (5-year OS: 52.9% *vs.* 60.1%, P=0.227; *Figure 1D*), implying that the expression level of *HDAC5* in tumor tissues is negatively correlated with prognosis.

To investigate the independent factors affecting the prognosis of GC, we included important clinicopathological data, such as sex, age, family history, tumor location, PD-L1, and TILs, in a univariate Cox regression model. The results (*Table 4*) revealed that *HDAC5* expression levels (P=0.008), $CD4^+$ T cells (P=0.029), $CD8^+$ T cells (P=0.040), family history (P=0.002), pT stage (P=0.010), pN stage (P<0.001), pM stage (P<0.001), pTNM stage (P<0.001), carcinoembryonic antigen (CEA) (P=0.004), carbohydrate antigen (CA)199 (P=0.025), and CA50 (P=0.035) had an impact on the prognosis of GC. Subsequently, a multivariate Cox regression model was constructed, and

Table 1 Clinicopathological features of 355 patients with GC

Clinicopathological features	Value
Age (years), median [range], mean ± standard error	64 [28, 91], 63.74±0.56
Sex, n (%)	
Male/female	256/99 (72.11/27.89)
Family history (GC), n (%)	
Yes/no/unknown	39/315/1 (10.99/88.73/0.28)
Smoking history, n (%)	
Yes/no/unknown	105/249/1 (29.58/70.14/0.28)
Drinking history, n (%)	
Yes/no/unknown	77/277/1 (21.69/78.03/0.28)
Weight loss, n (%)	
Yes/no/unknown	104/249/2 (29.30/70.14/0.56)
Tumor location, n (%)	
Proximal/distal/unknown	116/226/13 (32.68/63.66/3.66)
Borrmann type, n (%)	
I/II/III/IV/unknown	20/107/201/21/6 (5.63/30.14/56.62/5.92/1.69)
Lauren type, n (%)	
Intestinal/diffuse/mixed/unknown	197/108/43/7 (55.49/30.42/12.11/1.97)
Tumor size (cm), n (%)	
>5/≤5/unknown	170/180/5 (47.89/50.70/1.41)
Grade of differentiation, n (%)	
Undifferentiated + poorly differentiated/moderately-poorly differentiated/moderately + well differentiated/unknown	170/99/63/23 (47.89/27.89/17.75/6.48)
Pathological type, n (%)	
Adenocarcinoma/others	322/33 (90.70/9.30)
pT stage, n (%)	
T1 + T2/T3 + T4/unknown	30/318/7 (8.45/89.58/1.97)
pN stage, n (%)	
N0 + N1/N2 + N3/unknown	122/225/8 (34.37/63.38/2.25)
pM stage, n (%)	
M0/M1/unknown	326/22/7 (91.83/6.20/1.97)
pTNM stage, n (%)	
I/II/III/IV/unknown	17/51/258/22/7 (4.79/14.37/72.68/6.20/1.97)
AFP (ng/mL), n (%)	
≤8.1/>8.1/unknown	306/19/30 (86.20/5.35/8.45)

Table 1 (*continued*)

Table 1 (*continued*)

Clinicopathological features	Value
CEA (ng/mL), n (%)	
≤5/>5/Unknown	245/82/28 (69.01/23.10/7.89)
CA199 (U/mL), n (%)	
≤37/>37/unknown	233/94/28 (65.63/26.48/7.89)
CA724 (U/mL), n (%)	
≤6.9/>6.9/unknown	257/53/45 (72.39/14.93/12.68)
CA125 (U/mL), n (%)	
≤35/>35/unknown	279/14/62 (78.59/3.94/17.46)
CA50 (U/mL), n (%)	
≤25/>25/unknown	228/38/89 (64.23/10.70/25.07)

GC, gastric cancer; pT stage, pathological T stage; pN stage, pathological N stage; pM stage, pathological M stage; pTNM stage, pathological TNM stage; AFP, alpha fetoprotein; CEA, carcinoembryonic antigen; CA, carbohydrate antigen.

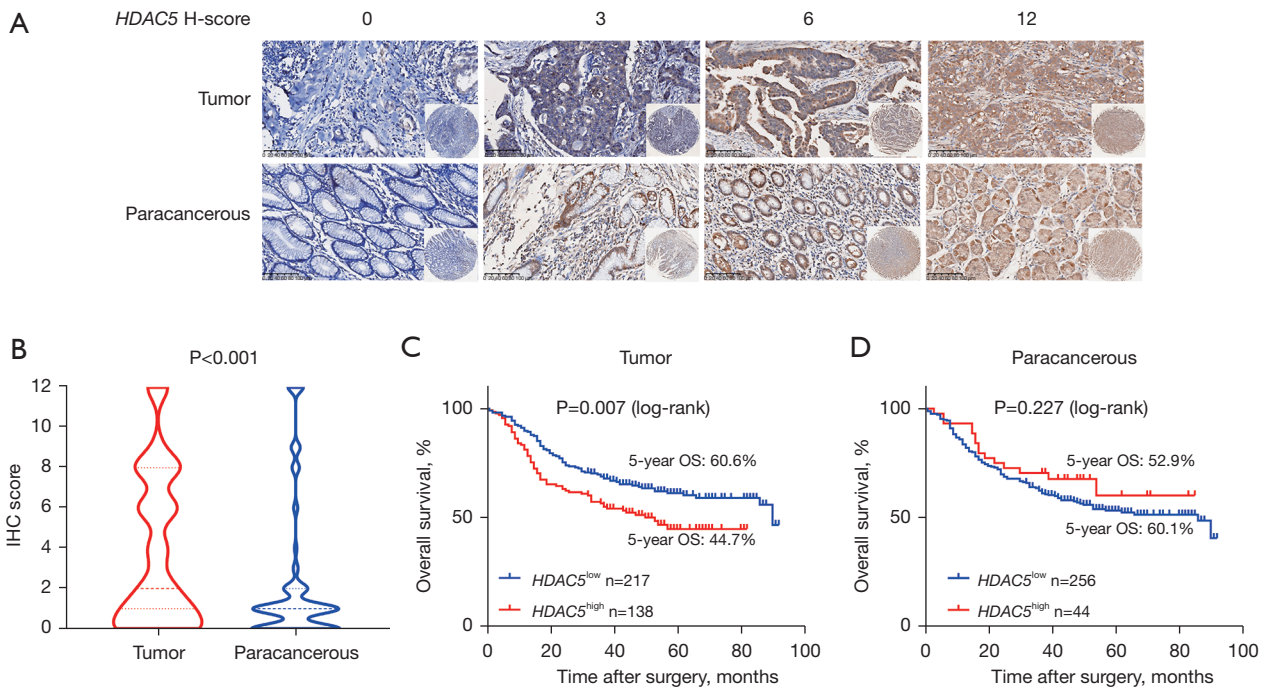

Figure 1 *HDAC5* is highly expressed in GC tumor tissues, and a high level of *HDAC5* predicts a worse prognosis in GC patients. (A) Representative images of *HDAC5* staining by IHC (×200-fold). (B) Differential expression of *HDAC5* in tumor and paracancerous tissues of GC. (C) The Kaplan-Meier OS curves of GC patients with different *HDAC5* levels in tumor tissues (log-rank test). (D) The Kaplan-Meier OS curves of GC patients with different *HDAC5* levels in paracancerous tissues (log-rank test). *HDAC5*, histone deacetylase 5; IHC, immunohistochemistry; OS, overall survival; GC, gastric cancer.

Table 2 Differential expression of *HDAC5* in GC

Variable	N	0 score	<6 scores	≥6 scores	Positive rate (>1) (%)	Positive rate (≥6) (%)
HDAC5	355	87	217	138	75.49	38.87

HDAC5, histone deacetylase 5; GC, gastric cancer.

Table 3 The differential expression of *HDAC5* in tumor tissues and paracancerous tissues

Parameters	N	*HDAC5* expression		Positive rate (%)	χ^2	P value
		Positive	Negative			
Tumor tissues	355	138	217	38.87	47.482	<0.001***
Paracancerous tissues	300	44	256	14.67		

***, P<0.001. *HDAC5*, histone deacetylase 5.

Table 4 Univariate Cox regression analysis of 355 GC patients

Parameters	Univariate Cox regression analysis		
	P value	HR	95% CI
HDAC5 expression			
Low *vs.* high	0.008**	1.542	1.122–2.119
Sex			
Male *vs.* female	0.819	0.959	0.673–1.367
Age (years)			
<65 *vs.* ≥65	0.235	1.210	0.883–1.658
Gastric history			
No *vs.* yes	0.002**	1.977	1.287–3.035
Smoking history			
No *vs.* yes	0.339	1.179	0.842–1.651
Drinking history			
No *vs.* yes	0.403	1.171	0.809–1.694
Weight loss			
No *vs.* yes	0.078	1.348	0.967–1.877
Tumor location			
Proximal *vs.* distal	0.096	0.754	0.541–1.051
Pathological type			
Adenocarcinoma *vs.* others	0.673	0.889	0.513–1.539
Borrmann type			
I + II *vs.* III + IV	0.108	1.324	0.940–1.864
Lauren type			
Intestinal *vs.* diffuse *vs.* mixed	0.289	1.126	0.904–1.403
Tumor size (cm)			
≤5 *vs.* >5	0.047*	1.381	1.004–1.899

Table 4 (*continued*)

Table 4 (*continued*)

Parameters	Univariate Cox regression analysis		
	P value	HR	95% CI
Grade of differentiation			
Undifferentiated + poorly differentiated *vs.* moderately-poorly + moderately + well differentiated	0.058	0.729	0.525–1.011
pT stage			
T1 + T2 *vs.* T3 + T4	0.010*	2.914	1.288–6.593
pN stage			
N0 + N1 *vs.* N2 + N3	<0.001***	3.632	2.366–5.576
pM stage			
M0 *vs.* M1	<0.001***	3.669	2.255–5.968
pTNM stage			
I + II *vs.* III + IV	<0.001***	2.518	1.501–4.225
AFP (ng/mL)			
≤8.1 *vs.* >8.1	0.054	1.754	0.991–3.104
CEA (ng/mL)			
≤5 *vs.* >5	0.004**	1.680	1.184–2.383
CA199 (U/mL)			
≤37 *vs.* >37	0.025*	1.482	1.052–2.090
CA724 (U/mL)			
≤6.9 *vs.* >6.9	0.483	1.163	0.763–1.772
CA125 (U/mL)			
≤35 *vs.* >35	0.060	1.918	0.974–3.778
CA50 (U/mL)			
≤25 *vs.* >25	0.035*	1.669	1.036–2.687
PD-L1			
Negative *vs.* positive	0.981	1.004	0.705–1.431
$CD3^+$ T cells			
Low *vs.* high	0.994	0.999	0.729–1.368
$CD4^+$ T cells			
Low *vs.* high	0.029*	0.704	0.513–0.965
$CD8^+$ T cells			
Low *vs.* high	0.040*	0.719	0.524–0.986

*, P<0.05; **, P<0.01; ***, P<0.001. GC, gastric cancer; *HDAC5*, histone deacetylase 5; pT stage, pathological T stage; pN stage, pathological N stage; pM stage, pathological M stage; pTNM stage, pathological TNM stage; AFP, alpha fetoprotein; CEA, carcinoembryonic antigen; CA, carbohydrate antigen; PD-L1, programmed cell death ligand-1; HR, hazard ratio; CI, confidence interval.

subsequent analysis revealed that *HDAC5* expression levels (P=0.036; HR =1.581; 95% CI: 1.031–2.426), $CD4^+$ T cell levels (P=0.012; HR =0.539; 95% CI: 0.334–0.872), $CD8^+$ T cell levels (P<0.001; HR =0.288; 95% CI: 0.144–0.577), and pTNM stage (P<0.001; HR =3.757; 95% CI: 1.790–7.886) were independent factors affecting GC prognosis (*Table 5*). Thus, both univariate and multivariate analyses showed that *HDAC5* was an independent prognostic factor for GC.

The expression of HDAC5 is correlated with age, gastric history, Lauren type, and pM stage

To further investigate the correlation between *HDAC5* expression levels and clinicopathological characteristics, we analyzed the correlation between groups using the chi-square test. The results (*Table 6*) revealed that the positive expression rate of *HDAC5* was higher in patients aged 65 years or older *vs.* those aged less than 65 years (44.19% *vs.* 33.88%, P=0.046) and in patients who smoked *vs.* those who did not (43.59% *vs.* 38.41%, P=0.001). The Lauren type was also found to be closely related to the expression level of *HDAC5*. Specifically, the expression level of *HDAC5* was significantly higher in mixed-type patients than in intestinal-type, and diffuse-type patients (P=0.042), with positive expression rates of 39.09%, 31.48%, and 53.49% in intestinal-type, diffuse-type and mixed-type patients, respectively. In addition, the expression level of *HDAC5* was significantly correlated with the pM stage, and the expression level of *HDAC5* was higher in the pM1 stage than in the pM0 (P=0.012). This suggests that the expression level of *HDAC5* is correlated with age, smoking history, Lauren type, and pM stage. However, other indicators, including sex, family history, tumor location and size, Borrmann type, grade of differentiation, pT stage, pN stage, CEA, and other common tumor markers, were not significantly correlated with *HDAC5* expression.

HDAC5 expression regulates the GC TIME

To explore the status of the TIME of GC patients, we performed an IHC assessment of 355 patients to determine the expression of TILs ($CD3^+$ T cells, $CD4^+$ T cells, $CD8^+$ T cells) and PD-L1 in tumor tissues. The median numbers of $CD3^+$ T cells, $CD4^+$ T cells, and $CD8^+$ T cells were used as the cutoff value to divide patients into high and low groups (*Figure 2A-2C*).

Patients were divided into positive and negative PD-L1 expression groups based on the CPS score (*Figure 2D*). Kaplan-Meier survival curves revealed that the numbers of $CD4^+$ T cells and $CD8^+$ T cells had a prognostic effect, and patients who exhibited high levels of $CD4^+$ T cells or $CD8^+$ T cells had a better prognosis (5-year OS: 60.6% *vs.* 48.9%, P=0.027; 60.6% *vs.* 48.6%, P=0.038; *Figure 2E,2F*). However, the $CD3^+$ T cell levels and PD-L1 expression levels had no significant effect on the prognosis of GC patients (5-year OS: 55.2% *vs.* 54.5%, P=0.994; 55.2% *vs.* 53.5%, P=0.981; *Figure 2G,2H*). This finding suggests that the levels of $CD4^+$ T cells and $CD8^+$ T cells are positively correlated with the prognosis of GC patients.

Furthermore, we further analyzed the relationship between *HDAC5* expression and TILs and PD-L1 in GC (*Table 7*). The correlations between *HDAC5* and the levels of $CD3^+$, $CD4^+$, and $CD8^+$ T cells were analyzed (*Figure 3A-3C*); we found that the levels of $CD3^+$ T cells (292.23±14.88 *vs.* 197.62±18.16, P<0.001), $CD4^+$ T cells (56.83±4.62 *vs.* 44.83±7.13, P<0.001), and $CD8^+$ T cells (177.08±10.22 *vs.* 108.79±10.30, P<0.001) were negatively correlated with the expression level of *HDAC5*. The analysis of PD-L1 expression between the high and low *HDAC5* expression groups (*Figure 3D*) showed that the positive rate of PD-L1 expression was higher in the high *HDAC5* expression group (20.7% *vs.* 37.0%, P=0.001), indicating that the expression of PD-L1 was positively correlated with the level of *HDAC5*.

In addition, we performed ssGSEA on the 375 STAD tissues downloaded from TCGA to identify their differential immune infiltrating cells. We plotted heat maps and violin plots (*Figure 4A,4B*) and observed that the expression level of *HDAC5* was significantly correlated with the levels of activated CD4 T cells (P<0.0001), activated CD8 T cells (P<0.0001), activated dendritic cells (P<0.001), CD56 bright natural killer cells (P<0.01), central memory CD8 T cells (P<0.05), gamma delta T cells (P<0.01), neutrophils (P<0.001), plasmacytoid dendritic cells (P<0.05), type 17 T helper cells (P<0.01), and type 2 T helper cells (P<0.05). The expression level of *HDAC5* was negatively correlated with the levels of immune infiltrating cells, except for plasmacytoid dendritic cells.

$HDAC5^{low}$ + $CD4^{high}$ status and $HDAC5^{low}$ + $CD8^{high}$ status predict a better prognosis

Based on the correlation between *HDAC5* and TILs and PD-L1, and Kaplan-Meier analysis confirming that TILs and PD-L1 influence the prognosis of GC, we next explored the prognostic impact of *HDAC5* on GC in

Table 5 Multivariate Cox regression analysis of 355 GC patients

Parameters	Multivariate Cox regression analysis		
	P value	HR	95% CI
HDAC5 expression			
Low *vs.* high	0.036*	1.581	1.031–2.426
$CD3^+$ T cells			
Low *vs.* high	0.001**	3.578	1.647–7.774
$CD4^+$ T cells			
Low *vs.* high	0.012*	0.539	0.334–0.872
$CD8^+$ T cells			
Low *vs.* high	<0.001***	0.288	0.144–0.577
PD-L1			
Negative *vs.* positive	0.538	1.156	0.728–1.836
Sex			
Male *vs.* female	0.773	0.937	0.603–1.457
Age (years)			
<65 *vs.* ≥65	0.047*	1.491	1.006–2.212
Gastric history			
No *vs.* yes	0.039*	1.754	1.029–2.991
Tumor size (cm)			
≤5 *vs.* >5	0.918	1.021	0.689–1.514
pTNM stage			
I + II *vs.* III + IV	<0.001***	3.757	1.790–7.886
CEA (ng/mL)			
≤5 *vs.* >5	0.599	1.122	0.732–1.720
CA199 (U/mL)			
≤37 *vs.* >37	0.223	1.420	0.808–2.495
CA50 (U/mL)			
≤25 *vs.* >25	0.348	1.365	0.713–2.614

*, P<0.05; **, P<0.01; ***, P<0.001. GC, gastric cancer; *HDAC5*, histone deacetylase 5; PD-L1, programmed cell death ligand-1; pTNM stage, pathological TNM stage; CEA, carcinoembryonic antigen; CA, carbohydrate antigen; HR, hazard ratio; CI, confidence interval.

combination with TILs or PD-L1. We divided the patients into four groups according to their $CD4^+$ T cell levels and *HDAC5* expression levels ($HDAC5^{low}$ + $CD4^{low}$ group; $HDAC5^{low}$ + $CD4^{high}$ group; $HDAC5^{high}$ + $CD4^{low}$ group; $HDAC5^{high}$ + $CD4^{high}$ group). Kaplan-Meier survival analysis (*Figure 5A*) demonstrated that the $HDAC5^{low}$ + $CD4^{high}$ group had the best prognosis (5-year OS: 69.5%), the $HDAC5^{high}$ + $CD4^{high}$ group had the worst prognosis (5-year OS: 39.9%), and the $HDAC5^{low}$ + $CD4^{low}$ and $HDAC5^{high}$ + $CD4^{low}$ groups had similar prognoses (5-year OS: 49.0% *vs.* 48.4%). The overall prognosis was significantly different among the four groups (P=0.004). The prognostic value analysis of *HDAC5* combined with $CD8^+$ T cells (*Figure 5B*) revealed that the $HDAC5^{low}$ + $CD8^{high}$ group had

Table 6 Correlation between *HDAC5* expression and clinicopathological characteristics in GC

Parameters	*HDAC5* expression		Total	Positive rate (%)	χ^2	P value
	Positive	Negative				
Age (years)					3.963	0.046*
≥65	76	96	172	44.19		
<65	62	121	183	33.88		
Sex					0.135	0.713
Female	40	59	99	40.40		
Male	98	158	256	38.28		
Family history					0.391	0.532
Yes	17	22	39	43.59		
No	121	194	315	38.41		
Unknown	1					
Smoking history					11.265	0.001**
Yes	55	50	105	52.38		
No	83	166	249	33.33		
Unknown	1					
Drinking history					0.072	0.788
Yes	29	48	77	37.66		
No	109	168	277	39.35		
Unknown	1					
Weight loss					0.008	0.931
Yes	40	64	104	38.46		
No	97	152	249	38.96		
Unknown	2					
Tumor location					2.106	0.147
Proximal	52	64	116	44.83		
Distal	83	143	226	36.73		
Unknown	13					
Borrmann type					0.116	0.734
I/II	48	79	127	37.80		
III/IV	88	134	222	39.64		
Unknown	6					

Table 6 (*continued*)

Table 6 (*continued*)

Parameters	*HDAC5* expression		Total	Positive rate (%)	χ^2	P value
	Positive	Negative				
Lauren type					6.355	0.042*
Intestinal	77	120	197	39.09		
Diffuse	34	74	108	31.48		
Mixed	23	20	43	53.49		
Unknown	7					
Tumor size (cm)					1.430	0.232
>5	72	98	170	42.35		
≤5	65	115	180	36.11		
Unknown	5					
Grade of differentiation					0.878	0.645
Undifferentiated + poorly differentiated	61	109	170	35.88		
Moderately-poorly differentiated	41	58	99	41.41		
Moderately + well differentiated	25	38	63	39.68		
Unknown	23					
pT stage					0.031	0.860
T1/T2	12	18	30	40.00		
T3/T4	122	196	318	38.36		
Unknown	7					
pN stage					1.274	0.259
N0/N1	52	70	122	42.62		
N2/N3	82	143	225	36.44		
Unknown	8					
pM stage					6.264	0.012*
M0	120	206	326	36.81		
M1	14	8	22	63.64		
Unknown	7					
pTNM stage					1.790	0.181
I/II	31	37	68	45.59		
III/IV	103	177	280	36.79		
Unknown	7					

Table 6 (*continued*)

Table 6 (*continued*)

Parameters	*HDAC5* expression		Total	Positive rate (%)	χ^2	P value
	Positive	Negative				
AFP (ng/mL)					0.015	0.903
≤8.1	117	189	306	38.24		
>8.1	7	12	19	36.84		
Unknown	30					
CEA (ng/mL)					0.189	0.664
≤5	92	153	245	37.55		
>5	33	49	82	40.24		
Unknown	28					
CA199 (U/mL)					0.236	0.627
≤37	91	142	233	39.06		
>37	34	60	94	36.17		
Unknown	28					
CA724 (U/mL)					0.165	0.685
≤6.9	99	158	257	38.52		
>6.9	22	31	53	41.51		
Unknown	45					
CA125 (U/mL)					0.021	0.885
≤35	105	174	279	37.63		
>35	5	9	14	35.71		
Unknown	62					
CA50 (U/mL)					0.433	0.510
≤25	95	133	228	41.67		
>25	18	20	38	47.37		
Unknown	89					

*, P<0.05; **, P<0.01. *HDAC5*, histone deacetylase 5; GC, gastric cancer; pT stage, pathological T stage; pN stage, pathological N stage; pM stage, pathological M stage; pTNM stage, pathological TNM stage; AFP, alpha fetoprotein; CEA, carcinoembryonic antigen; CA, carbohydrate antigen.

the best prognosis (5-year OS: 62.4%), the *HDAC5*^{high} + CD8^{low} group had the worst prognosis (5-year OS: 40.6%), and the remaining two groups had similar prognoses (5-year OS: 57.2% *vs.* 53.6%); the difference in prognosis among the groups was statistically significant (P=0.023).

Although the survival analysis of CD3$^+$ T cells and PD-L1 showed no significant correlation with prognosis, based on the correlation between these factors and *HDAC5*, we next performed an integrated prognostic value analysis of *HDAC5* expression coupled with CD3$^+$ T cells (*Figure 5C*). The prognosis was relatively good in the *HDAC5*^{low} + CD3^{low} and *HDAC5*^{low} + CD3^{high} groups, with similar 5-year OS rates (60.6% and 60.5%, respectively). Also, the *HDAC5*^{high} + CD3^{low} (5-year OS: 48.3%) and *HDAC5*^{high} + CD3^{high} (5-year OS: 37.9%) groups had worse prognoses, and there was a significant difference in the OS among the four groups (P=0.031). Finally, a survival analysis was performed according to the expression of *HDAC5*

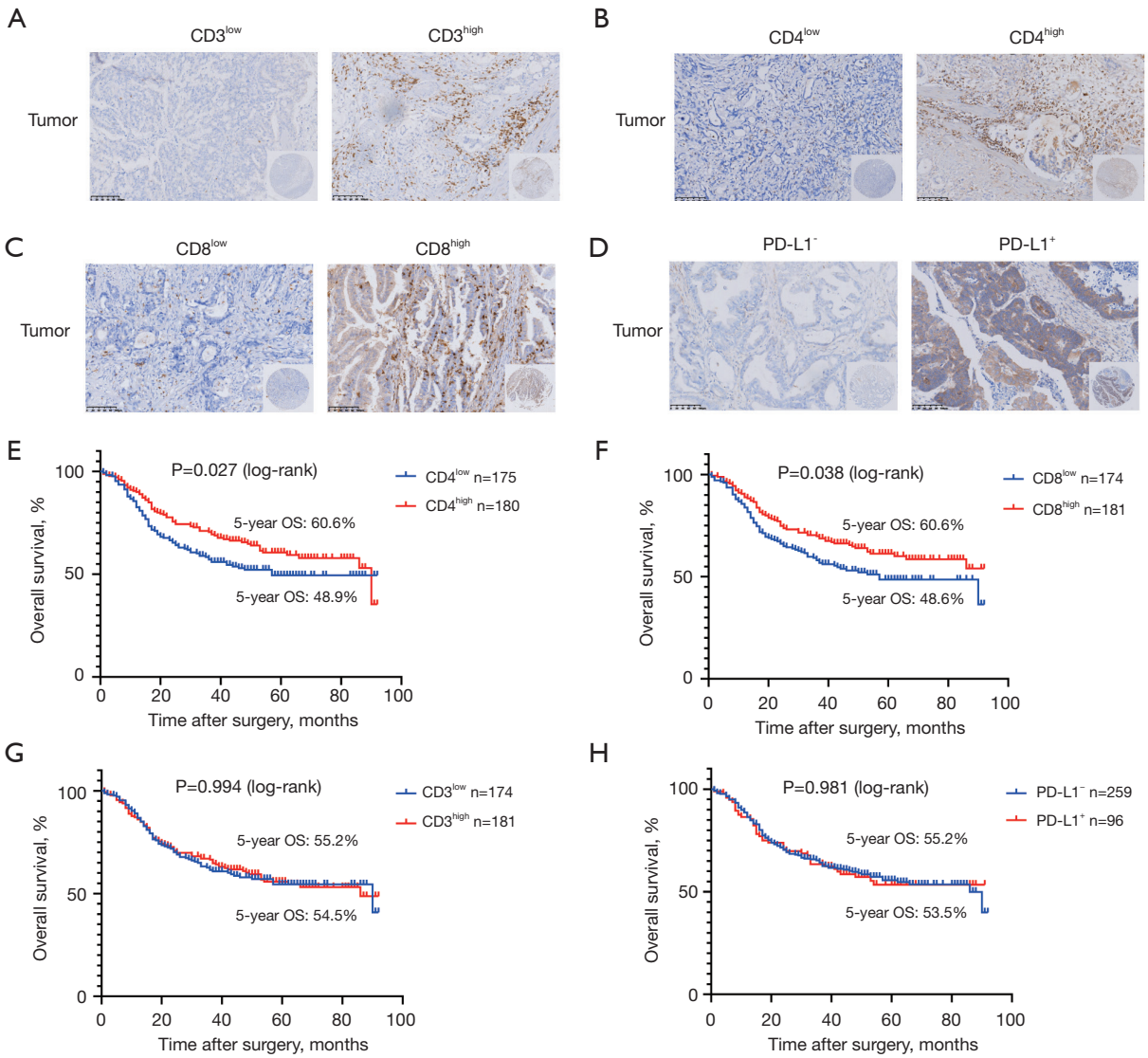

Figure 2 TILs and PD-L1 were correlated with the prognosis of GC. Representative images (×200-fold) of CD3$^+$ T cells (A), CD4$^+$ T cells (B), CD8$^+$ T cells (C) and PD-L1 (D) staining by IHC. The Kaplan-Meier OS curves of GC patients with different CD4$^+$ T cell (E), CD8$^+$ T cell (F), CD3$^+$ T cell (G) and PD-L1 (H) levels in tumor tissues (log-rank test). OS, overall survival; PD-L1, programmed cell death ligand-1; TILs, tumor-infiltrating lymphocytes; GC, gastric cancer; IHC, immunohistochemistry.

Table 7 The correlation between *HDAC5* expression and CD3, CD4, CD8 and PD-L1 expression in GC

Parameters	*HDAC5 vs.* CD3	*HDAC5 vs.* CD4	*HDAC5 vs.* CD8	*HDAC5 vs.* PD-L1
χ^2/Z value	−6.055	−3.840	−5.980	11.247
P	<0.001***	<0.001***	<0.001***	0.001**

**, P<0.01; ***, P<0.001. *HDAC5*, histone deacetylase 5; PD-L1, programmed cell death ligand-1; GC, gastric cancer.

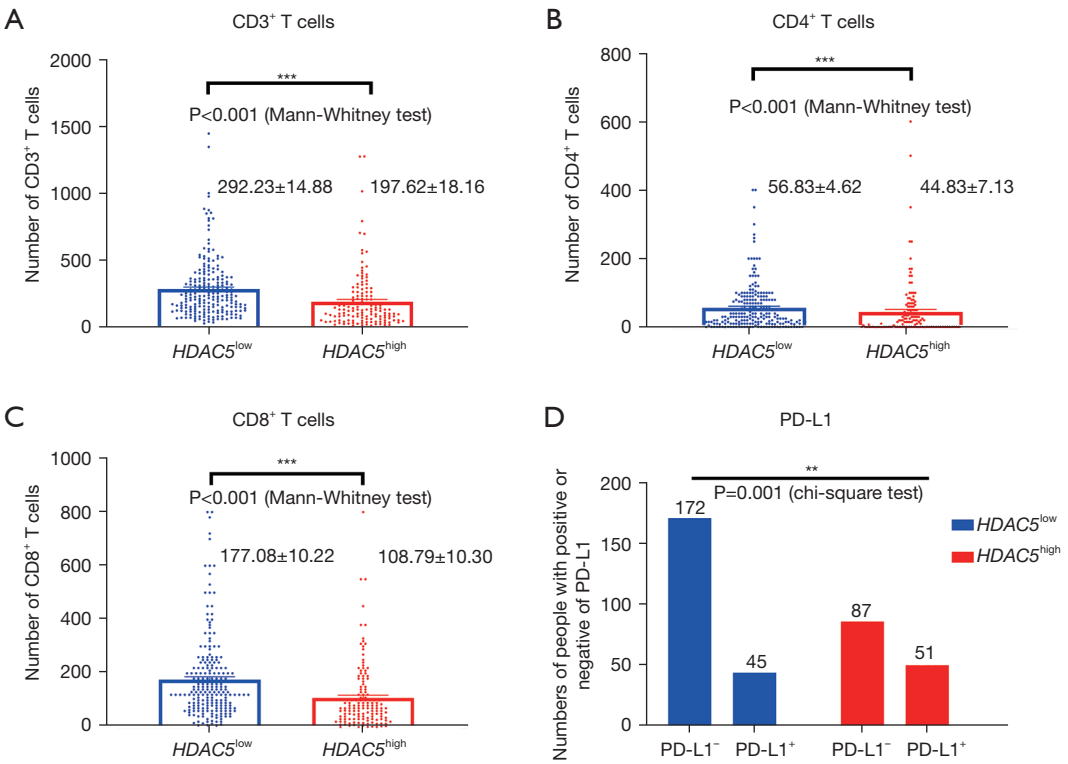

Figure 3 *HDAC5* expression was highly negatively correlated with TILs levels and positively correlated with PD-L1 expression. (A) Correlation between *HDAC5* expression and CD3$^+$ T cell levels. (B) Correlation between *HDAC5* expression and CD4$^+$ T cell level. (C) Correlation between *HDAC5* expression and CD8$^+$ T cell levels. (D) Correlation between *HDAC5* expression and PD-L1 expression. **, P<0.01; ***, P<0.001. *HDAC5*, histone deacetylase 5; PD-L1, programmed cell death ligand-1; TILs, tumor-infiltrating lymphocytes.

and PD-L1 (*Figure 5D*) and found that the *HDAC5*^{low} + PD-L1$^-$ and *HDAC5*^{low} + PD-L1$^+$ groups had good and similar prognoses (5-year OS: 59.4% *vs.* 64.9%), while the *HDAC5*^{high} + PD-L1$^-$ and *HDAC5*^{high} + PD-L1$^+$ groups had relatively worse prognoses (5-year OS: 46.5% *vs.* 42.6%), and there was a statistically significant OS difference among the groups (P=0.046).

Discussion

Histone acetylation and deacetylation are among the most common post-translational modifications. *HDACs* maintain a dynamic balance between acetylation and deacetylation (20), thereby regulating cell proliferation, apoptosis, metastasis, and cell cycle progression and affecting histone properties and their biological functions (21,22). Previous studies have demonstrated that *HDAC5* is differentially expressed in tumor tissues. Patani *et al.* performed RNA extraction and reverse transcription in 127 BC tissues and 33 normal tissues and used quantitative real-time PCR (qRT-PCR) to determine the transcription levels of *HDAC* genes and investigate the expression differences. The expressions of *HDACs*, including *HDAC5*, were found to be significantly different in BC tissues compared with normal tissues, and *HDAC5* expression was significantly upregulated in BC tissues (23). A study by Fan *et al.* reported that the mRNA and protein levels of *HDAC5* were determined in HCC tissues and cells using qRT-PCR and protein blotting, and similarly, both the mRNA and protein levels of *HDAC5* were found to be upregulated (24).

The expressions of *HDACs* in GC have also been investigated, and some studies have reported that the expression levels of *HDAC1*, *HDAC2*, and *HDAC4* are upregulated in cancer tissues (25-27), while some other family members, such as HDAC3, have been shown to exhibit decreased expression in cancer tissues (28). However, studies on *HDAC5* in GC are lacking, and the results of available studies are highly variable; therefore,

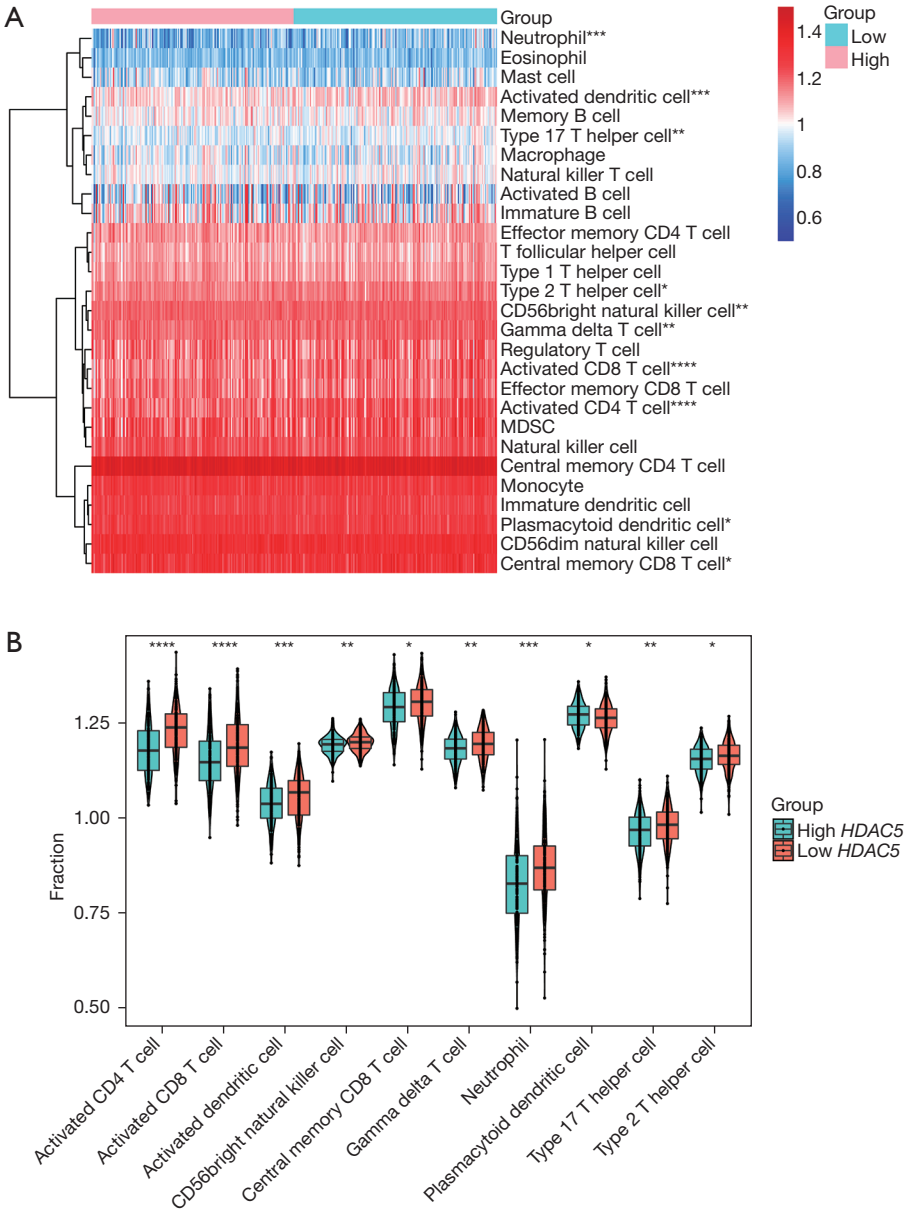

Figure 4 The expression of *HDAC5* was closely related to TILs levels according to TCGA. Heat map (A) of *HDAC5*-associated infiltrating cells and violin plot (B) of *HDAC5*-associated differentially infiltrating immune cells. *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001. *HDAC5*, histone deacetylase 5; TILs, tumor-infiltrating lymphocytes; TCGA, The Cancer Genome Atlas.

further investigation is needed. The expression of *HDAC5* in GC was first reported by Orenay-Boyacioglu *et al.*, who assessed the expressions of *HDACs* by qRT-PCR in 28 GC tumor tissues and 20 normal tissues. They reported that the expression level of *HDAC5* was downregulated in tumor tissues compared with control tissues (29). However, Chen *et al.* used gene expression profiling interaction analysis (GEPIA) to explore the mRNA levels of *HDACs* and found that the expression levels of *HDAC5* were not significantly different in GC tissues compared with normal tissues (30). Interestingly, we determined the expression levels of *HDAC5* in tumor tissues (n=355) and paracancerous tissues (n=300) by IHC and observed that the expression levels of *HDAC5* were significantly increased

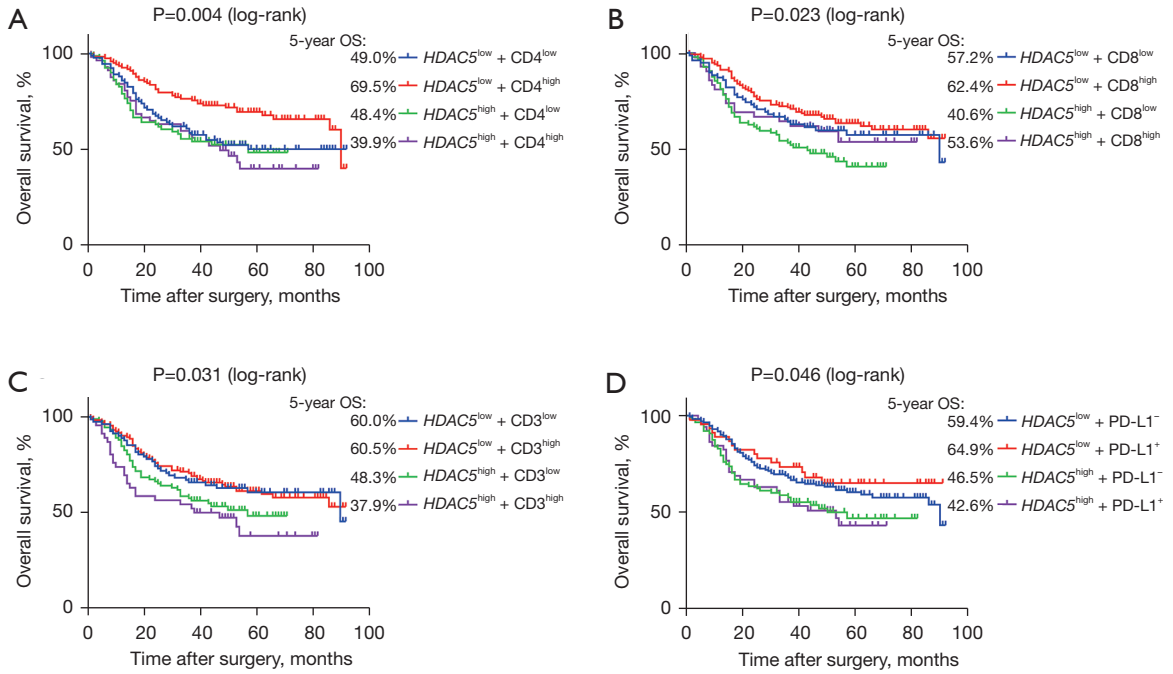

Figure 5 *HDAC5*^{low} + CD4^{high} and *HDAC5*^{low} + CD8^{high} status predict a better prognosis. (A) Kaplan-Meier OS curves of GC patients with different levels of *HDAC5* and CD4^{+} T cells (log-rank test). (B) Kaplan-Meier OS curves of GC patients with different levels of *HDAC5* and CD8^{+} T cells (log-rank test). (C) Kaplan-Meier OS curves of GC patients with different levels of *HDAC5* and CD3^{+} T cells (log-rank test). (D) Kaplan-Meier OS curves of GC patients with different levels of *HDAC5* and PD-L1 (log-rank test). *HDAC5*, histone deacetylase 5; OS, overall survival; PD-L1, programmed cell death ligand-1; GC, gastric cancer.

in tumor tissues (P<0.001). This suggests that the value of *HDAC5* in GC still needs to be further confirmed by more studies.

HDAC5 plays an important role in cancer development and is a potential prognostic marker (31), and has been shown to have an impact on the prognosis of different tumors. Zhou *et al.* showed that overexpression of *HDAC5* adversely affected the OS and progression-free survival (PFS) of ovarian cancer patients (32). Similarly, Klieser *et al.*'s study in pNET confirmed *HDAC5* as a predictor of poor clinical outcomes (9). However, Zhang *et al.* investigated *HDAC5* in astrocytoma and found that *HDAC5* expressed at high levels was indicative of a better prognosis (33), suggesting that this gene exhibits different prognostic values for different cancer types. There are few studies on the prognosis of *HDAC5* in GC patients, and only Chen *et al.* have studied the effect of *HDAC5* on the prognosis of GC; their study confirmed that a high *HDAC5* expression level was closely associated with poor prognosis (30). Similarly, we performed a survival analysis for high and low *HDAC5* expression and identified a negative correlation between GC prognosis and *HDAC5* expression level (P=0.007). Meanwhile, the univariate and multivariate analyses confirmed that *HDAC5* was an independent factor affecting the prognosis of patients.

Currently, there are few studies demonstrating a correlation between *HDAC5* expression and clinicopathological features. Only Chen *et al.* used GEPIA to explore the mRNA levels of *HDACs* in GC and reported that the expression levels of *HDAC5* in GC were correlated with Lauren type, clinical stage, lymph node status, treatment, and human epidermal growth factor receptor 2 status (30). Our study reached some of the same conclusions, as we found a significant correlation between *HDAC5* expression levels and Lauren type (P=0.042) and pM stage (P=0.012). In addition, we observed that *HDAC5* expression levels were also markedly correlated with age (P=0.046) and smoking status (P=0.001). These results suggest that *HDAC5* is more likely to be highly expressed in seniors, smokers, those with mixed Lauren type, and those with distant metastases.

A growing number of studies have confirmed that the activation of *HDACs* can affect PD-L1 expression in various

types of cancer. In pancreatic ductal adenocarcinoma, Zhou *et al.* analyzed the correlation between *HDAC5* and PD-L1 expression using TMAs and found that PD-L1 expression was negatively correlated with *HDAC5* expression (P=0.0028) (18). Woods *et al.* demonstrated that *HDAC* inhibitors can alter immunogenicity and enhance antitumor immune responses in melanoma, and that class I *HDAC* inhibitors can upregulate PD-L1 (34). Thus far, no studies have reported on the effect of *HDAC5* expression on PD-L1 expression in GC. We measured the expressions of *HDAC5* and PD-L1 in 355 GC tissues by IHC and found that the expression of *HDAC5* was positively correlated with PD-L1 (P=0.001). Further clarification of the role of TILs might contribute to a comprehensive understanding of the TIME, which may help guide personalized immunotherapy, and TILs are currently a hot topic in cancer immunotherapy research (35).

The relationship between *HDAC* expression and immune cell infiltration remains debatable. Xiao *et al.* designed experiments showing that *HDAC5*-negative mice attenuated the suppressive function of regulatory T cells (Treg cells), while the silencing of *HDAC5* inhibited the switch from $CD4^+$ T cells to Tregs and suppressed IFN-γ production in $CD8^+$ T cells (36). We identified a correlation between the *HDAC5* expression level and the numbers of $CD3^+$ T cells, $CD4^+$ T cells, and $CD8^+$ T cells, and determined that all of these were negatively correlated with the *HDAC5* expression level. We also analyzed the TIME of 375 GC tissues in TCGA and observed that the expression level of *HDAC5* was negatively correlated with activated CD4 T cells, activated CD8 T cells, and other types of immune cells. This finding indicated that *HDAC5* may play an important regulatory role in the TIME of GC.

Previous research has shown that TILs (including $CD3^+$, $CD4^+$, and $CD8^+$ T cells) are associated with a good prognosis in GC (37-39). However, the prognostic value of PD-L1 in GC is still controversial. Some studies have confirmed that PD-L1 expression is associated with a good prognosis (40-42), while others have confirmed that PD-L1 expression is either associated with a poor prognosis or does not have a prognostic value (43,44). We investigated the prognostic significance of PD-L1 and TILs and found that high expression levels of $CD4^+$ T cells and $CD8^+$ T cells were associated with a good prognosis. Our univariate and multivariate analyses confirmed that $CD4^+$ T cells and $CD8^+$ T cells were independent factors affecting the prognosis of patients. However, PD-L1 and $CD3^+$ T cells did not exhibit a significant effect on prognosis in this study.

Finally, through combined survival analysis, we also found that the $HDAC5^{low}$ + $CD4^{high}$ and $HDAC5^{low}$ + $CD8^{high}$ groups had the best prognosis, with 5-year OS rates of 69.5% and 62.4%, respectively. Based on the negative correlation between *HDAC5* expression level and prognosis and the positive correlation between high expression levels of $CD4^+$ T cells and $CD8^+$ T cells, we can conclude that the joint action of *HDAC5* with $CD4^+$ T cells and $CD8^+$ T cells has some influence on the prognosis of GC. This confirms that *HDAC5* may be involved in the regulation of the GC tumor microenvironment, but the specific mechanism still requires further investigation. In conclusion, *HDAC5* can be considered a potential diagnostic marker for GC and a potential target for immunotherapy.

Conclusions

HDAC5 is highly expressed in tumor tissues and is an independent factor affecting the prognosis of GC. Additionally, *HDAC5* can regulate the TIME of GC and is a potential target for immunotherapy.

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Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-4325/rc

Data Sharing Statement: Available at https://atm.amegroups.com/article/view/10.21037/atm-22-4325/dss

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of Cancer Hospital of the University of Chinese Academy of Sciences (Zhejiang Cancer Hospital) (No. IRB-2021-431) and informed consent was taken from all the patients.

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