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Original Article

Decreased expression of histone deacetylase 10 predicts poor prognosis of gastric cancer patients

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Abstract: Aberrant expression of histone deacetylase (HDACs) was associated with carcinogenesis and progression of various tumors. However, the association of HDAC10 with clinical outcomes in gastric cancer patients is unclear. Thus, the objective of the current study was to evaluate the association of expression level of HDAC10 with clinicopathologic factors and prognosis of patients with gastric cancer. The expression level of HDAC10 in 179 paraffin-embedded gastric cancer tissue specimens was examined by immunohistochemistry (IHC). As a result, we found that expression of HDAC10 in gastric cancer was significantly decreased in gastric cancer tissues as compared with adjacent tissues (51.4% vs. 87.3%, $P < 0.001$). HDAC10 expression was significantly correlated with gender ($P = 0.023$), tumor size ($P = 0.015$), histological grade ($P = 0.009$), tumor invasion ($P = 0.033$), lymph node metastatic status ($P = 0.019$) and tumor stage ($P = 0.004$), but not correlated with age and lauren classification (all $P > 0.05$). Kaplan-Meier survival curves showed that the overall survival rate was significantly lower in the patients with low expression of HDAC10 compared with those patients with high HDAC10 ($P < 0.001$). Moreover, multivariate analysis revealed that HDAC10 expression was an independent prognostic factor for gastric cancer patients ($P = 0.001$). These results suggest that HDAC10 expression could see as a prognosis marker for gastric cancer patients.

Keywords: Histone deacetylase 10, gastric cancer, immunohistochemistry, prognosis

Introduction

Gastric cancer is the fourth most common type of malignant tumor and the second common cause of cancer-related deaths worldwide [1]. The treatment included surgery, chemotherapy, radiation therapy and target therapy [2]. In spite of improvement in early diagnosis and therapy, the prognosis was still poor [3]. The outcome of patients is difficult to predict with classical histological classification because of heterogeneity [4]. Therefore, to improve the prognosis of gastric cancer, better understanding of molecular mechanism of tumor progression and the new therapies based on these mechanisms are required [5]. Tumor progression involves the activation of oncogenes and inactivation of tumor suppressor genes [6].

Aberrant expression of histone deacetylase (HDACs) was associated with carcinogenesis and progression of various tumor [7-10]. The main function of HDACs is to modify the chromatin structure and regulate gene transcription [11, 12]. The HDAC family contains 18 proteins, which are grouped into classes I-IV based on their structure and homology. Classes I, II, and IV contain 11 family members, which are called classical HDACs, whereas the seven classes III family members are referred to as sirtuins [13]. HDAC10 is a class IIb HDAC, including HDAC6 and HDAC10. HDAC10, unlike HDAC6 that has two tandem deacetylase domains, has one deacetylase (DAC) domain and one additional catalytically inactive leucine-rich domain (LRD) [14]. Various studies confirmed that HDAC10 paly role in tumor suppressor. For instance, it been reported that HDAC10 suppressed cervical cancer metastasis through inhibition of matrix metalloproteinase (MMP) 2 and 9 expression [15].

However, to the best of our knowledge, no previous studies report the correlation between HDAC10 and prognosis of primary gastric cancer. Therefore, in this study, the expression of

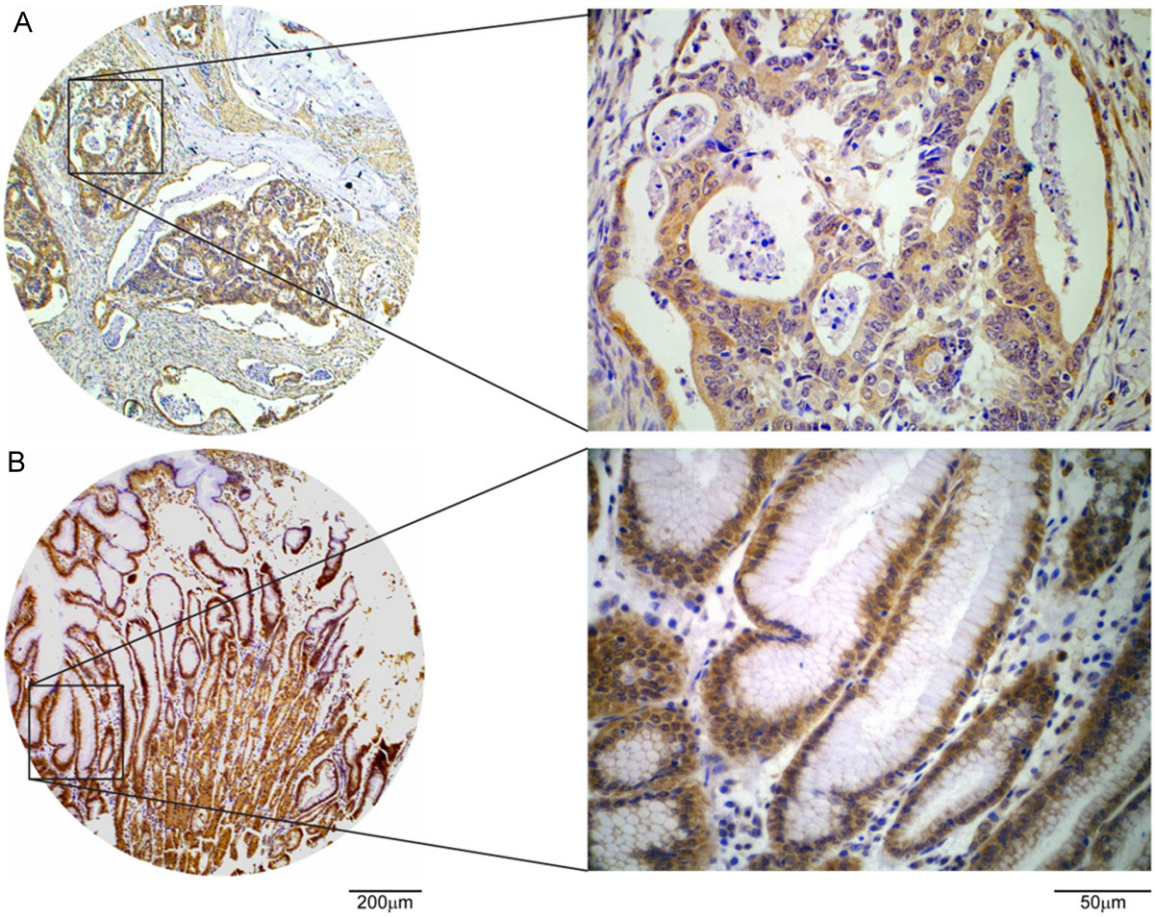

Figure 1. Decreased expression of HDAC10 in gastric cancer compared with adjacent tissue. A: gastric cancer (× 50, × 200), B: adjacent tissue (× 50, × 200).

Table 1. Decreased expression of HDAC10 in gastric cancer

	Cases	HDAC10 expression		*P value*
		negative	positive	
Gastric cancer	179	87	92	< 0.001
Adjacent Tissue	79	10	69	

HDAC10 was evaluated using immunohistochemistry. In addition, we identified the relationship between HDAC10 expression and clinicopathological characteristics as well as patients' prognosis.

Methods

Ethics statement

The institutional review board or ethics committee at each participating institution approved the study protocol. Written informed consent was obtained from each participant before data collection.

Patients

A total of 179 patients with primary gastric cancer from the pathology archives of the Yangzhong People's Hospital were include between 2002 and 2004. Patients who met the following criteria: (1) diagnosis of gastric cancer identified by histopathological examination; (2) surgical history that included gastrostomy and lymphadenectomy; (3) with complete follow-up data; (4) no preoperative treatment, such as chemotherapy or radiotherapy; (5) no death in the perioperative period. Clinicopathological characteristics including age, sex, histologic type and pathologic stage were collected by reviewing medical charts and pathology records. Each tumor samples was assigned histological grade based on the World Health Organization (WHO) classification criteria. All patients were staged using the

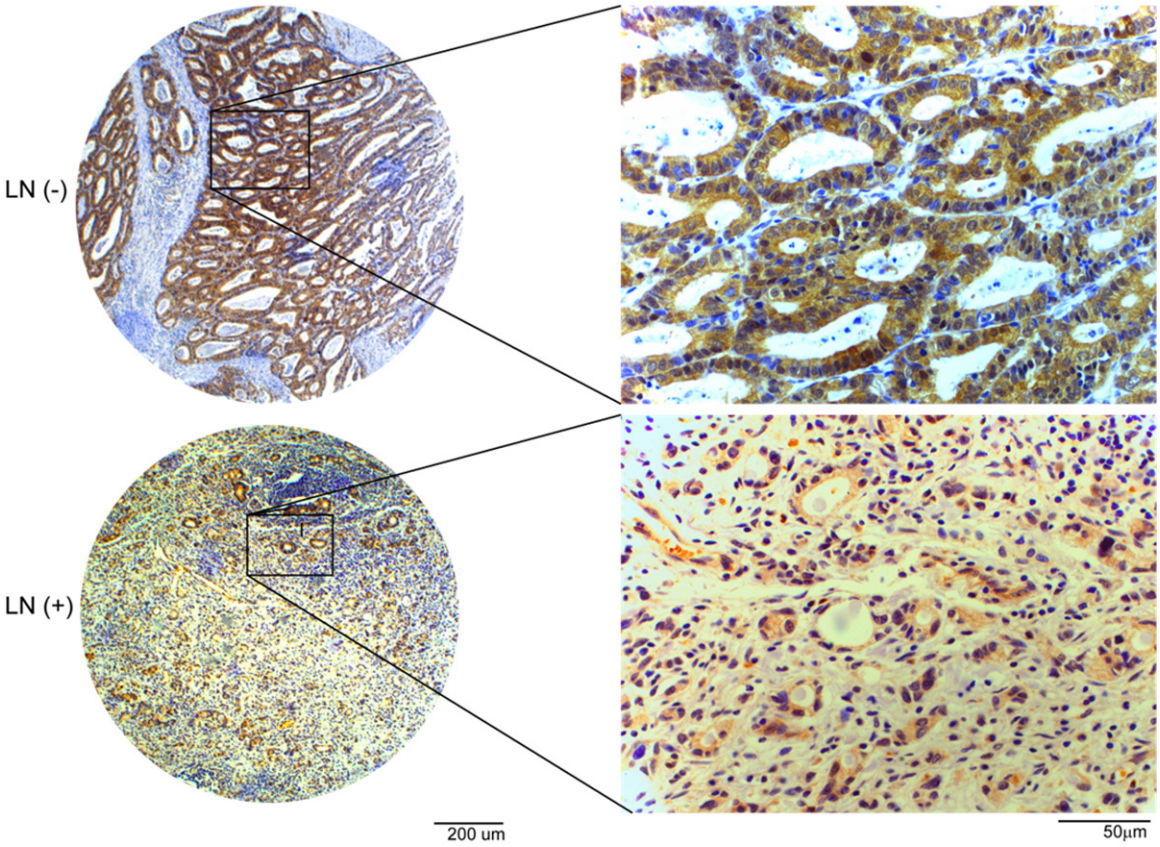

Figure 2. Different expression of HDAC10 in patients with or without lymph node metastatic status. Expression of HDAC10 was lower in tissue with lymph node metastatis.

7th edition of the International Union Against Cancer (UICC) Tumor-Node-Metastasis (TNM) staging system [11].

Among these patients, 124 were men and 55 were women, with an age range between 32 and 80 years old (a median of 59 years old). Patients were followed from the date of surgery until death, or censored on 31 April, 2014. The follow-up period ranged from 1 to 156 (a median of 52 months).

Tissue microarray construction

In brief, H & E-stained sections were made from primary tumor blocks to define two representative tumor regions and adjacent normal gastric tissues. Representative tumor regions were defined as tumor solid areas containing more than 75% cancer cells without necrosis. Normal gastric tissues were randomly selected adjacent to a tumor with distance of more than 5 cm, avoiding the bleeding areas. Tissue cylinders (1.5 mm in diameter) were then punched from the defined regions of the block using a tissue microarrayer (Gentury, IL, USA) and brought into recipient paraffin blocks. Two sets of three paraffin-embedded tissue microarray (TMA) blocks were made. Sections of the resulting TMA blocks were transferred to glass slides. There were a total of two sets of TMA, containing 179 tumor tissue spots and 79 adjacent normal gastric tissue spots each, available for this study (collaborating with Shanghai Biochip, Shanghai, China).

Immunohistochemistry

The tissue sections were deparaffinized with dimethylbenzene and rehydrated with grade ethanol. Then, heat-mediated antigen retrieval was carried out in pressure cooker with buffer containing 0.01M sodium citrate-hydrochloric acid (PH 6.0) for 4 min. After rinsing with PBS, endogenous peroxidase was blocked by 3% hydrogen peroxide. The sections were incubated with a rabbit polyclonal antibody against HDAC10 (dilution 1:1000; Abcam, Cambridge, UK) at 4°C overnight and then with horseradish peroxidase (HRP) (Gene Tech GTVision III

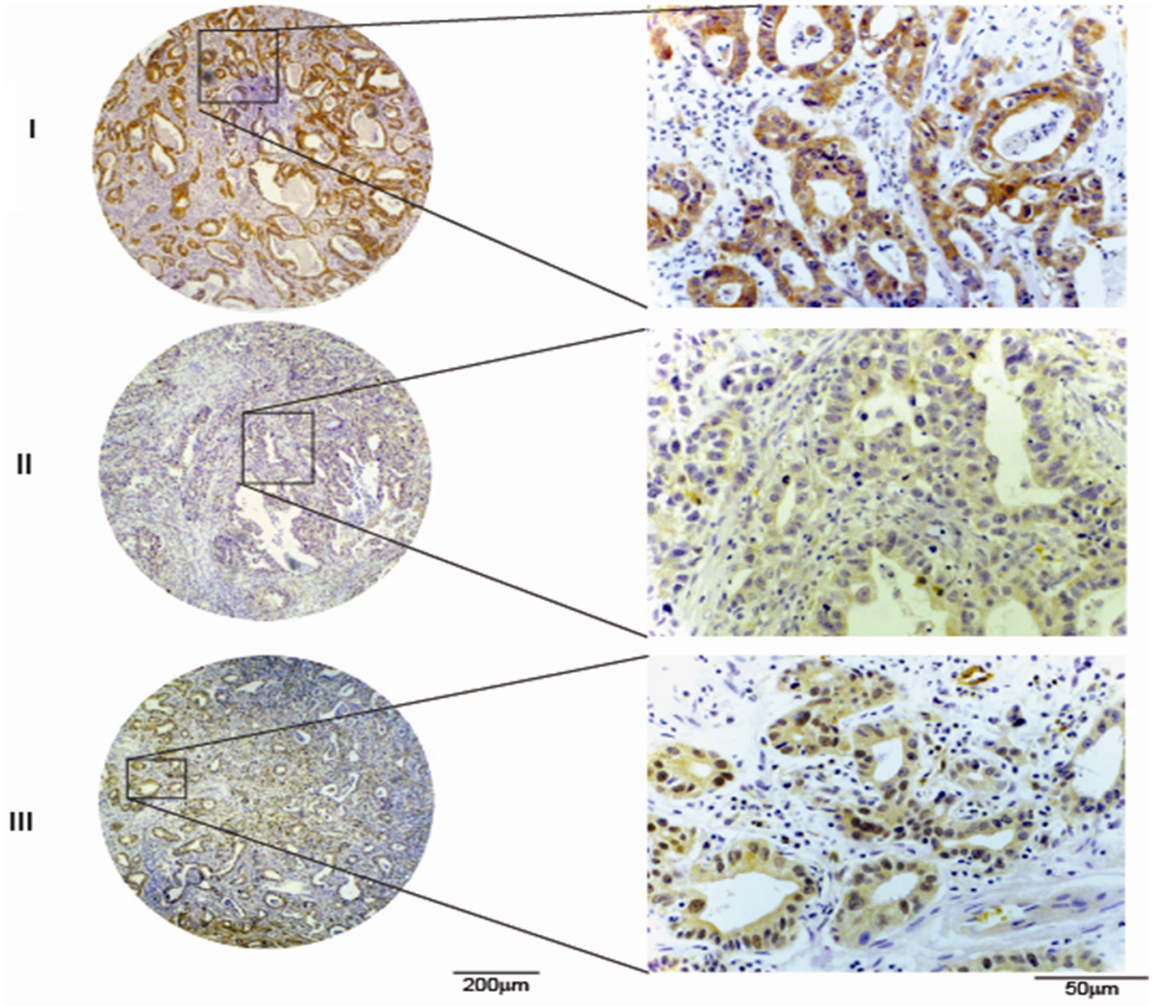

Figure 3. Different expression of HDAC10 in patients with different stage. Decreased expression of HDAC10 in tissue with advanced stage.

Detection Kit, Shanghai, China) at room temperature for 40 min.

Following washing with PBS for 3 times, the signal was detected with 3, 3'-diaminobenzidine (DAB) solution. For negative controls, adjacent sections were carried out as described above, with exception that they were incubated overnight at 4°C in blocking solution without primary antibody.

Score of immunohistochemistry

Evaluation was carried out dependently by two investigators (Lei Wang and Weihua Jiang), blinded to the patients outcomes. The staining was scored according to the staining intensity and percentage. Staining intensity was scored as 0 (negative), 1 (weakly positive), 2 (moderately positive), and 3 (strongly positive). The percentages of cells were scored into five categories: 0 (0%), 1 (1-25%), 2 (26%-50%), 3 (51-75%), and 4 (76-100%). The final staining scores were calculated by staining intensity × percentages of stained cells.

Statistical analysis

Categorical data were analyzed using χ^2 statistics. The probability of survival by different subgroups was calculated using the Kaplan-Meier method, and statistical significance was analyzed by using the log-rank test. Multivariate analysis was carried out by using the Cox proportional hazards model with adjustment for covariates to identify primary prognostic indicators that were independently associated with survival. All statistics were two-sided, at a sig-

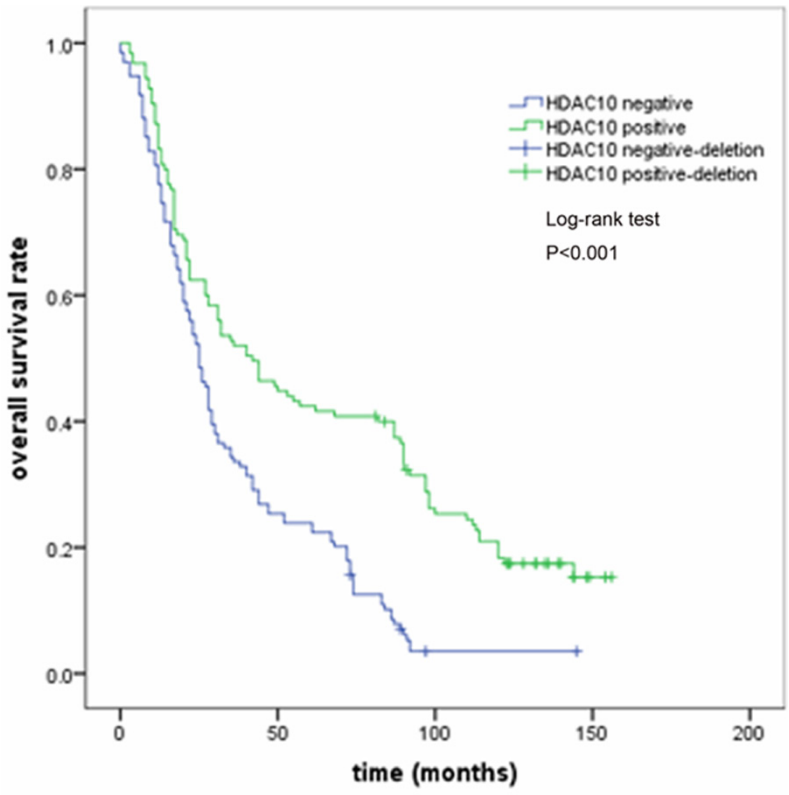

Figure 4. Kaplan-Meier survival curves of gastric cancer patients based on the HDAC10 expression level. Patients with low HDAC10 expression level had a significantly poorer survival than those with high HDAC10 expression level (P < 0.001, log-rank test).

nificant level of $P < 0.05$, by using the SPSS statistical software package for Windows (release 13.0, SPSS, Inc., Chicago, IL, USA).

Results

Decreased expression of HDAC10 in gastric cancer tissue compared with adjacent tissue

To obtain insight into the effect and prognostic value of HDAC10 expression in gastric cancer, paraffin-embedded tissues were examined using immunohistochemistry. HDAC10 was found to be largely localized in the cytoplasm and occasionally in nucleus. HDAC10 expression was different between gastric cancer and adjacent tissue. In brief, for tumor samples, 92 cases (51.4%) displayed positive, while 87.3% of adjacent tissue samples showed positive (**Table 1**; **Figure 1**).

Correlation between HDAC10 expression and clinicopathlogical characteristics

Then, we investigated association of HDAC10 expression and clinicopathological features. HDAC10 expression was found to be significantly reduced in patients with large tumors ($P = 0.015$) and poorly differentiation ($P = 0.009$). Decreased HDAC10 expression was observed significantly more frequently in tumors with deeper invasion ($P = 0.033$), tumors with a high lymph node metastatic status ($P = 0.019$) and cases with advanced stage ($P = 0.004$). Expression was also assessed to be significantly reduced in female patients ($P = 0.023$), however, it was not correlated with age ($P = 0.389$), Lauren classification ($P = 0.495$) (**Figures 2**, **3**; **Table 2**).

Association between HDAC10 expression and prognosis

The prognostic effect of HDAC10 on the survival rate of gastric cancer was investigated by comparing the survival rate of patients with or without HDAC10 expression using Kaplan-Meier survival curves and log-rank test. The 1-, 3-, 5-, 10-year overall survival rates in this cohort were 83.8%, 42.5%, 32.8% and 10.5%, respectively. The 5-year overall survival rates in patients with or without HDAC10 expression were 42.4% and 23.9%. And the 10-year overall survival rates were 18.3% and 3.5%. The overall survival of patients with negative HDAC10 expression was significantly shorter than those with negative expression ($P < 0.001$) (**Figure 4**).

Univariate and multivariate analysis

Univariate analysis showed that lymph metastasis ($P < 0.001$), tumor invasion ($P < 0.001$), tumor stage ($P < 0.001$), age ($P = 0.04$), tumor size ($P < 0.001$), lauren classification ($P = 0.027$), histological grade ($P = 0.001$), nodal status ($P < 0.001$) were significantly related to overall survival. Then, Cox regression analyzed models was constructed to compare the prognostic significance of HDAC10. Results con-

Table 2. Correlation between HDAC10 expression and clinicopathologicfeatures of patients with gastric cancer

		HDAC10 expression		
Variables	Numbers	negative	positive	*P value*
Age (years)				0.389
< 55	44	24	20	
≥ 55	135	63	72	
Gender				0.023
Male	124	53	71	
Female	55	34	21	
Tumor size (cm)				0.015
≤ 5	99	40	59	
> 5	80	47	33	
Histological grade				0.009
Well differentiated	25	8	17	
Moderately differentiated	85	36	49	
Poorly differentiated	69	43	26	
Lauren classification				0.495
Intestinal	115	52	63	
Diffuse	39	21	18	
Mixed	25	14	11	
Lymph metastasis				0.014
Yes	113	63	50	
No	66	24	42	
Tumor Invasion (T)				0.033
T1	14	4	10	
T2	27	8	19	
T3	136	74	62	
T4	2	1	1	
Nodal status (N)				0.019
N0	64	23	41	
N1	102	58	44	
N2	9	3	6	
N3	1	1	0	
Metastasis Status (M)				0.737
M0	177	86	91	
M1	2	1	1	
TNM stage				0.004
I-II	74	26	48	
III-IV	102	59	43	

firmed that tumor invasion (*P = 0.004*), lymph metastasis (*P = 0.001*) and HDAC10 expression (*P = 0.001*) were independent predictors of the overall survival of patients with gastric cancer (**Table 3**).

Discussion

To the best of our knowledge, this study represents the first observation about aberrant expression of histone deacetylase 10 (HDAC10) and relation with prognosis in gastric cancer. We found that expression of HDAC10 was decreased in gastric cancer tissue compared with adjacent tissue (51.4% vs. 87.3%). In addition, decreased expression of HDAC10 was associated with poor prognosis in univariate analysis; furthermore, multivariate analysis showed that HDAC10 expression was independent prognosis factor in gastric cancer.

Tumor progression arises as a consequence of a series of cellular events, including deregulation of proliferation, resistance to apoptosis, enhanced cell motility, augmented angiogenesis and disordered microenvironment, resulting in tumor formation, invasion and metastasis [16]. Inevitably, any cellular events in tumor progression involve deregulation of oncogene and tumor suppressor gene [17], not only resulting from genetic alteration, but epigenetic modification [6]. Histone acetylation is important part of epigenetic modification, which is regulated by histone deacetylase (HDAC) and histone acetyltransferase (HAT) [18, 19].

According to molecular structure, enzymatic activity, localization and expression pattern, HDACs are divided into four classes: class I (HDAC1, 2, 3 and 8 have homology to yeast RPD3); class IIa (HDAC4, 5, 7 and 9 have homology to yeast HDA1); class IIb (HDACs 6 and 10 have two catalytic sites) and class IV (HDAC11, has conserved residues shared with both class I and II deacetylases) [20]. It has been established that many members of the HDAC family play key role in promoting carcinogenesis. Class I HDACs can promote cell cycles and cancer cell proliferation [21]. Muller BM, et al. found that HDAC2 and HDAC3 were strongly expressed in subgroups of tumor with features of a more aggressive tumor type [22]. Overexpression of HDAC2 predicts unfa-

Table 3. Univariate and multivariate survival analysis of clinic-pathologic variables in gastric cancer patients

	Univariate analysis			Multivariate analysis		
Variables	HR	95% CI	*P* value	HR	95% CI	*P* value
HDAC10 expression (positive vs. negative)	23.9	0.38-0.66	< 0.001	11.37	0.46-0.81	0.001
Age (years) (< 55 vs. ≥ 55)	4.24	0.54-0.99	0.040			
Gender (male vs. female)	0.66	0.85-1.48	0.416			
Tumor size (cm) (> 5 cm vs. ≤ 5)	15.34	1.31-2.27	< 0.001			
Histological grade (poorly/moderately/well)	12.0	1.17-1.75	0.001			
Lauren classification (mixed/diffuse/intestinal)	4.9	1.02-1.43	0.027			
Lymph metastasis (yes vs. no)	34.0	1.79-3.23	< 0.001	10.72	1.24-2.39	0.001
Tumor Invasion (T) (T4/T3/T2/T1)	25.9	1.55-2.68	< 0.001	8.34	1.15-2.10	0.004
Nodal status (N) (N3/N2/N1/N0)	31.9	1.45-2.16	< 0.001			
TNM stage (III-IV vs. I-II)	34.7	1.77-3.11	< 0.001			

vorable prognosis in human gallbladder carcinoma [23].

In our study, HDAC10, a member of class II, is deceased in gastric cancer tissue compared with adjacent tissue by immunohistochemistry. HDAC10 expression was found to be significantly reduced in patients with large tumors and poorly differentiation. Decreased HDAC10 expression was observed significantly more frequently in tumors with deeper invasion, tumors with a high lymph node metastatic status and cases with advanced stage. Furthermore, high expression of HDAC10 predicts better patient outcome. It is consistent with previous study. Hirotaka et al. found that reduced expression of class II HDAC gene, especially HDAC10, was significantly associated with poor prognosis and independent predictor of poor prognosis in non-small cell lung cancer [9]. Song et al. confirmed that HDAC10 suppressed expression of matrix metalloproteinase (MMP) 2 and 9, thus to inhibit cervical cancer cell invasion and metastasis. While, reduced expression of HDAC10 in cervical cancer was observed and associated with lymph node metastasis [15].

HDACs can regulate the binding of transcription factors to DNA, change the structure of chromatin and serve as a signal to regulate the expression of downstream gene [24]. Study found that HDAC10 bind to MMP 2 and -9 promoter regions, reduce the histone acetylation level and inhibit the binding of RNA polymerase II to these regions.

As a result, our study together with other studies demonstrates that different class of HDACs may play different role in tumor progression and prognosis. HDACs inhibitors are widely considered as promising anticancer therapeutics [25, 26]. One key consideration is to develop isoform-specific HDAC inhibitors that do not target metastasis-suppressing HDACs (e.g. HDAC10).

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Disclosure of conflict of interest

None.

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