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Expression and prognostic analyses of HDACs in human gastric cancer based on bioinformatic analysis

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Abstract

Gastric cancer (GC) is a common cancerous tumor, and is the third leading cause of cancer mortality worldwide. Although comprehensive therapies of GC have been widely used in clinical set ups, advanced gastric cancer carries is characterized by poor prognosis, probably due to lack of effective prognostic biomarkers. Mammalian histone deacetylase family, histone deacetylases (HDACs), play significant roles in initiation and progression of tumors. Aberrant expression of HDACs is reported in many cancer types including gastric cancer, and may serve as candidate biomarkers or therapeutic targets for GC patients.

Gene Expression Profiling Interactive Analysis was used to explore mRNA levels of HDACs in GC. Kaplan–Meier plotter was used to determine the prognostic value of HDACs mRNA expression in GC. Genomic profiles including mutations of HDACs were retrieved from cBioPortal webserver. A protein–protein interaction network was constructed using STRING database. GeneMANIA was used to retrieve additional genes or proteins related to HDACs. R software was used for functional enrichment analyses.

Analysis of mRNA levels of HDAC1/2/4/8/9 showed that they were upregulated in GC tissues, whereas HDAC6/10 was downregulated in GC tissues. Aberrant expression of HDAC1/3/4/5/6/7/8/10/11 was all correlated with prognosis in GC. In addition, expression levels of HDACs were correlated with different Lauren classifications, and clinical stages, lymph node status, treatment, and human epidermal growth factor receptor 2 status in GC.

The findings of this study showed that HDAC members are potential biomarkers for diagnosis or prognosis of gastric cancer. However, further studies should be conducted to validate these findings.

Abbreviations: GC = gastric cancer, GEPIA = Gene Expression Profiling Interactive Analysis, GO = gene ontology, HDACis = HDAC inhibitors, HDACs = histone deacetylases, HER2 = human epidermal growth factor receptor 2, KEGG = Kyoto Encyclopedia of Genes and Genomes, OS = overall survival.

Keywords: bioinformatic analysis, gastric cancer, HDAC, prognostic value

1. Introduction

Gastric cancer (GC) originates from epithelium mucosa of the stomach, and is the fifth most prevalent diagnosed cancer and the third leading cause of cancer-related mortality worldwide.[1] The 5-year survival of gastric cancer ranges approximately from 57% to 18% in different countries, owing to its high malignancy, invasion, and recurrence.[2,3] Currently, clinicians rely mainly on American Joint Committee on Cancer staging system for prognostic prediction of gastric cancer.[4] Despite advances in comprehensive therapies of GC including surgery, chemotherapy, radiation, and target therapy, the prognostic outcome remains poor. Therefore, there is need to explore novel diagnostic and prognostic indicators, to improve treatment of GC patients.

Mammalian histone deacetylase (HDAC) family comprises 18 members, which are subdivided into 4 classes based on their sequence homology and cofactor specificity. These classes include Class I histone deacetylases (HDACs) (HDAC1/2/3/8, homologous with yeast Rpd3), Class II HDACs (HDAC4/5/6/7/9/10, homologous with yeast Hda1), Class III HDACs or Sirtuins (SIRT1–7, homologous with yeast Sir2), and Class IV HDACs (HDAC11, homologous with both class I and class II).[5–8] Class I, II, and IV are referred to as "classical" HDACs, and their activities are Zn^+-dependent, whereas Class III HDACs activity is NAD^+-dependent (this family will not be subject of discussion in

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The datasets generated during and/or analyzed during the current study are publicly available.

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this article).[9,10] Previous studies report that HDACs are involved in proliferation, apoptosis, invasion, and migration of various cancer cell types, including gastric cancer cells, thus modulating carcinogenesis and cancer progression.[11–13] In addition, aberrant expression of HDACs is reported in gastric cancer, and may serve as candidate biomarkers and therapeutic targets for GC patients.[12,14–18] The potential role of HDACs in gastric cancer has been receiving increasing attention.

Bioinformatic analyses are widely used in genomics as a result of advances in microarray technology and establishment of online databases. In this study, bioinformatic analyses were performed using online databases to explore expression profiles and prognostic values of HDACs in GC.

2. Materials and methods

2.1. Gene expression analysis

mRNA levels of HDACs in GC were analyzed using Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia.cancer-pku.cn). GEPIA contains RNA sequence expression data of 9736 tumors and 8587 normal tissue samples.[19] Expression levels of HDACs between tumor and normal tissues were compared using Student's *t* test. Expression of HDACs in different pathological stages of GC was compared using *F* test. Statistical significance was defined as $P < .01$ and fold change > 2.

2.2. Prognosis analysis

Kaplan–Meier Plotter (http://www.kmplot.com) was used to analyze the prognostic value of HDACs mRNA expression. Kaplan–Meier Plotter contains gene expression data and survival information of 1440 clinical GC patients. To explore the overall survival (OS) of GC patients, samples were divided into low and high expression groups based on median mRNA levels, with a hazard ratio (HR) with 95% confidence intervals and log-rank *P* value. Kaplan–Meier survival analysis was then performed on the 2 groups. Log-rank *P* value $< .05$ was used to show statistical significance. Univariate cox analysis was conducted with adjustments to different Lauren classification, clinical stages, lymph node status, treatment, and human epidermal growth factor receptor 2 (HER2) status of GC.

2.3. Analysis of gene alteration and associated network construction

To explore gene alterations of HDACs in GC patients, genomic profiles including mutations were obtained from cBioPortal webserver for Cancer Genomics (http://www.cbioportal.org). Protein–protein interaction network analysis was performed on HDAC members using STRING database (https://string-db.org/), to explore potential interactions between HDACs. GeneMANIA tool (http://www.genemania.org) was used to retrieve additional genes or proteins related to HDACs.

2.4. Functional enrichment analysis

Functional enrichment analysis of HDACs were performed using gene ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway were conducted and visualized in R software using "org.Hs.eg.db," "clusterProfiler," "pathview," "Goplot," and "ggplot2" packages. Level of significance was set at *P*-value $< .05$.

2.5. Ethical statement

All data were retrieved from open-access databases, not directly from patients or animals. Therefore, ethical approval was not necessary.

3. Results

3.1. Gene expression of HDACs in patients with GC

mRNA expression levels of HDACs in GC tissues were compared with those in normal tissues using GEPIA tool, which contains 408 GC samples and 211 normal gastric samples. Analysis showed differential expression of HDACs in GC tissues compared with normal tissues (Fig. 1). mRNA levels of HDAC1,

Figure 1. The relative level of HDACs in STAD (GEPIA). GEPIA = Gene Expression Profiling Interactive Analysis; HDACs = histone deacetylases.

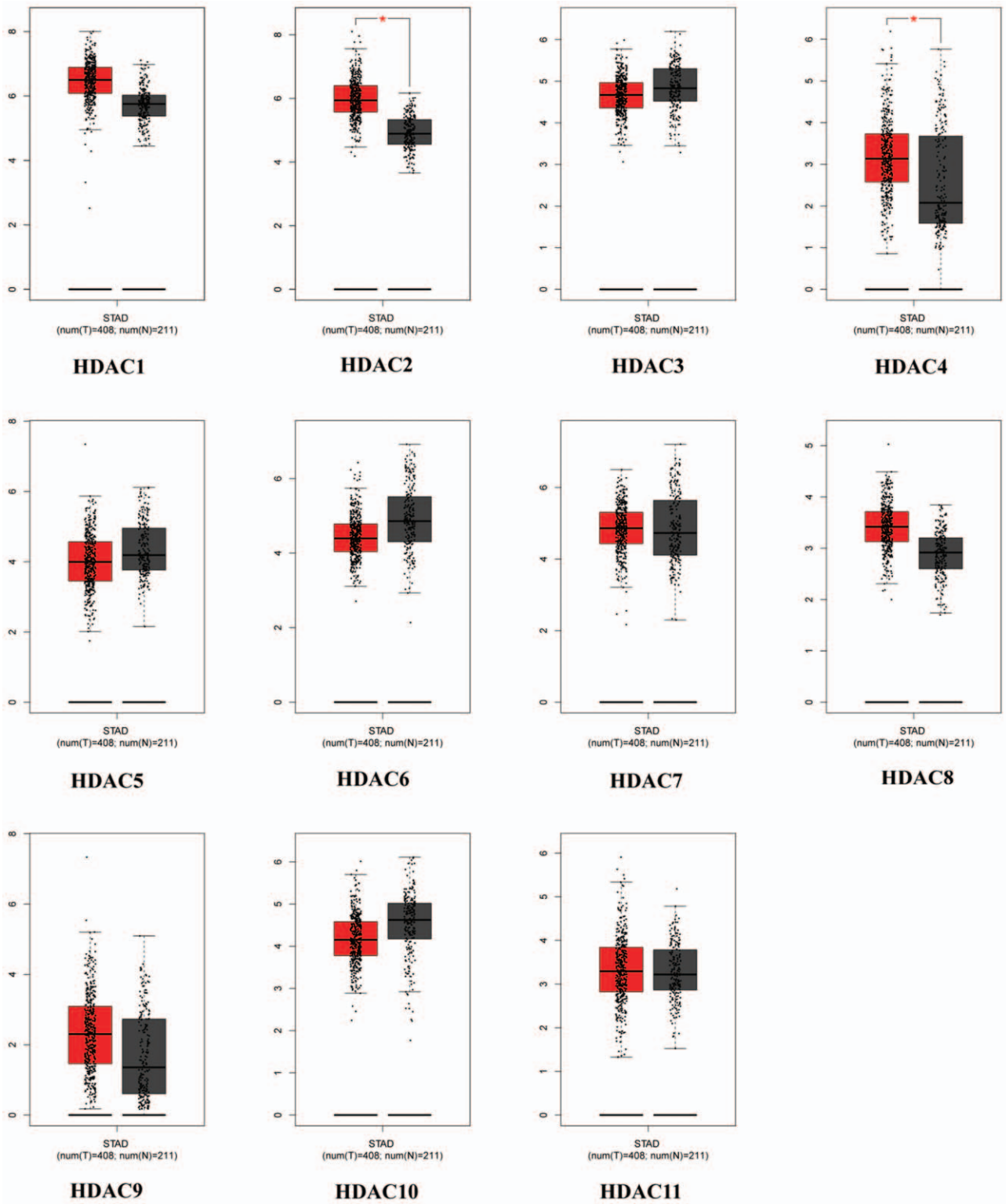

Figure 2. The mRNA expression of HDACs in GC patients (GEPIA). GC = gastric cancer; GEPIA = Gene Expression Profiling Interactive Analysis; HDACs = histone deacetylases.

HDAC2, HDAC4, HDAC8, and HDAC9 were upregulated in GC tissues compared with normal tissues (Fig. 2). On the other hand, analysis of mRNA expression showed that HDAC6 and HDAC10 were downregulated in GC tissue compared with normal tissues. Notably, box plots showed significance increase in mRNA expression levels of HDAC2 and HDAC4 mRNA levels. Analysis showed no differences for mRNA expression levels of the HDAC3/5/7/11 between GC and normal tissues. Moreover, expression of HDACs in different pathological stages of GC was explored. Expression levels of HDAC9 and HDAC11

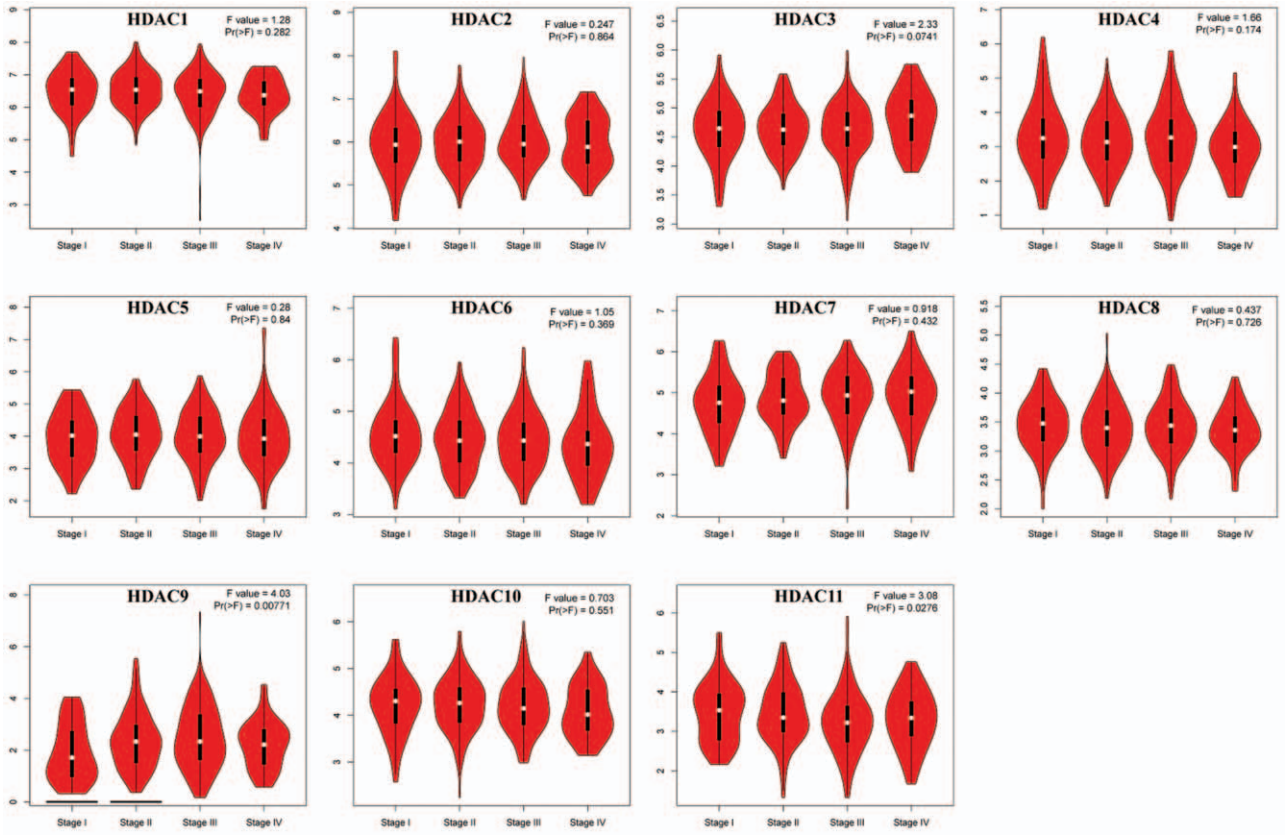

Figure 3. The expression of HDACs in different tumor stage of GC patients (GEPIA). GC = gastric cancer; GEPIA = Gene Expression Profiling Interactive Analysis; HDACs = histone deacetylases.

varied significantly in different pathological stages, whereas expression of other HDAC members in various stages was not significantly different across pathological stages (Fig. 3).

3.2. Prognostic value of HDACs in gastric cancer

Correlation analysis of the expression of HDACs and prognosis of GC patients was performed using Kaplan–Meier plotter. The findings showed that low mRNA expression of HDAC3 was correlated with favorable OS in all gastric cancer cases. Similarly, low expression levels of HDAC4/5/6/7/8/10/11 were correlated with favorable OS for GC patients (Fig. 4). GC patients with high HDAC1 mRNA levels were predicted to have a good prognosis, whereas mRNA expression levels of HDAC2 and HDAC9 were not statistically correlated with OS of GC patients (Fig. 4).

Furthermore, prognostic significance of HDACs in different Lauren classification of GC, including intestinal, diffuse, and mixed type was determined. GC patients with decreased mRNA expression of HDAC3, HDAC5, HDAC6, HDAC7, HDAC8, HDAC9, and HDAC10 showed longer OS, whereas patients with decreased HDAC1 and HDAC4 mRNA expression showed shorter OS in intestinal gastric cancer (Fig. 5). Low HDAC4/5/6/7/8/9/10/11 mRNA expression levels or increased HDAC1 mRNA levels were correlated with good prognosis in GC patients of diffuse type (Fig. 6).

3.3. Prognostic value of HDACs in GC patients with different clinicopathological characteristics

Clinical stages, lymph node status, treatment, and HER2 status of patients with GC were compared to explore prognostic values of HDACs in patients with different clinicopathological characteristics. Low HDAC3/6 mRNA levels were correlated with favorable OS in stage 1 gastric cancer patients, whereas low expression of HDAC3/5/6/7/8/9 was correlated with good prognosis in stage 2 patients (Table 1). In stage 3 GC, low mRNA expression levels of HDAC3/5/6/7/8/10/11 were correlated with longer OS, whereas low HDAC1 mRNA expression levels were correlated with shorter OS. In addition, high expression of HDAC1/3 or low expression of HDAC4/5/7/9/10 was correlated with a good prognosis of stage 4 GC patients.

Analysis of lymph node negative GC patients showed that high HDAC1 mRNA levels, and low HDAC6/9 mRNA levels were significantly correlated with a good prognosis. A positive lymph node status was correlated with increased overall survival in patients with high expression level of HDAC1 or low expression levels of HDAC4/5/6/7/8/10/11, as shown in Table 2.

Prognostic values of HDACs in GC patients with 2 different treatments, including surgery alone and 5 FU based adjuvant were analyzed (Table 3). Analysis of the surgery-alone group, patients with decreased mRNA expression levels of HDAC4/5/7/8/9 or increased mRNA expression level of HDAC1 showed better OS. High HDAC2/9 mRNA or low HDAC1/3/6/11

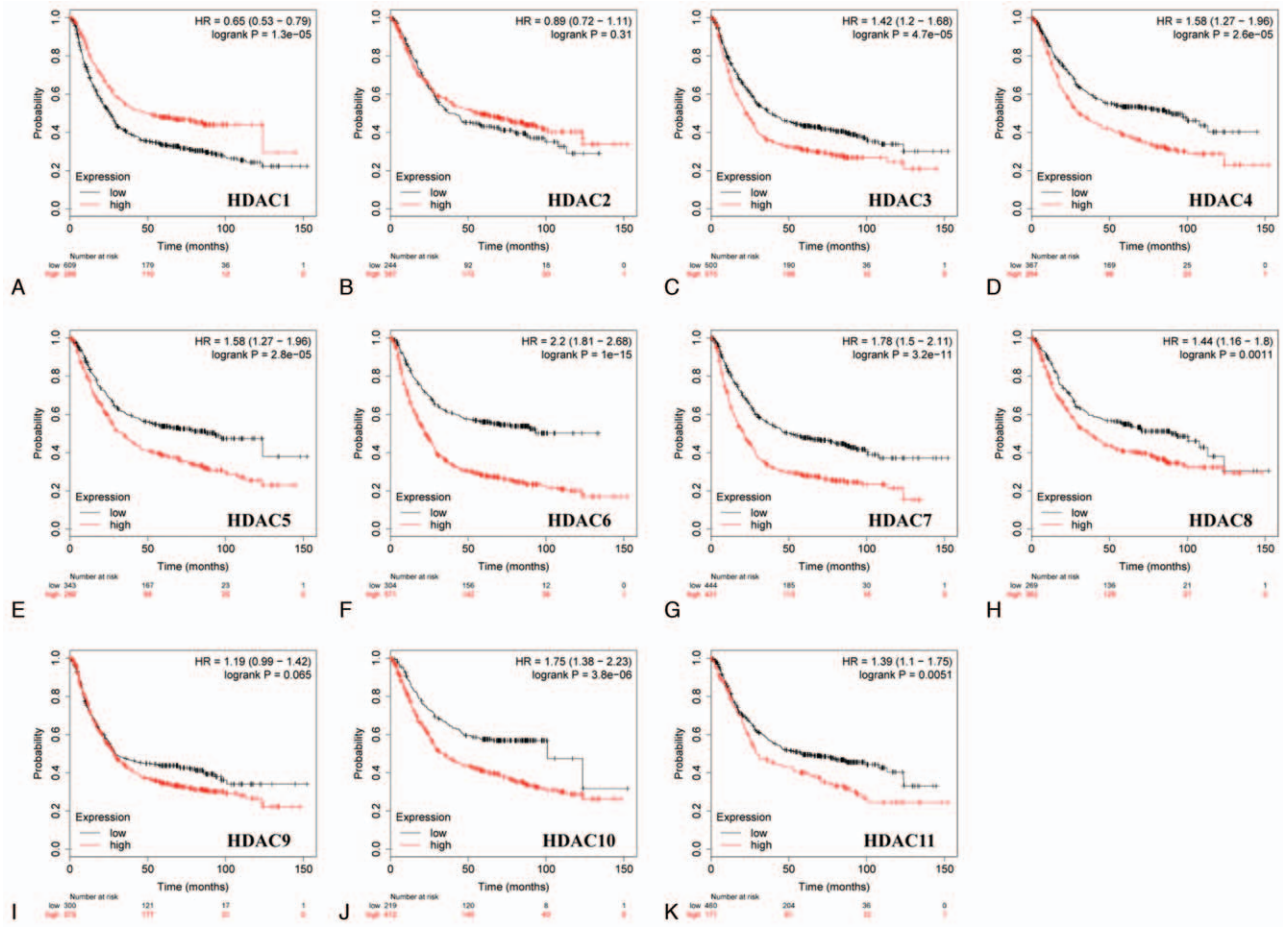

Figure 4. Correlation of HDAC mRNA expression with OS in all GC patients (Kaplan–Meier plotter). GC = gastric cancer; OS = overal survival.

mRNA levels were correlated with longer OS for GC patients treated with 5 FU based adjuvant.

Analysis based on HER2 status showed that expression levels of all HDACs, except for HDAC2, were correlated with overall survival in HER2-negative GC patients (Table 4). Low expression of HDAC3/4/5/6/7/8/9/10/11 or high HDAC1 expression was correlated with good prognosis. In HER2-positive GC patients, low mRNA levels of HDAC3/6/7/8/10 were correlated with favorable OS.

3.4. Genetic alterations and functional prediction of HDACs in GC

Genetic alterations of HDACs in GC patients were analyzed using cBioPortal tool. A total of 147 samples out of 708 (21%; data not shown) with stomach adenocarcinoma showed altered expression levels of at least 1 HDAC. Percentages of alterations in HDACs among 5 GC datasets ranged from 1.3% to 6% for individual genes (HDAC1, 2.1%; HDAC2, 3%; HDAC3, 1.3%; HDAC4, 6%; HDAC5, 4%; HDAC6, 2.8%; HDAC7, 2.2%; HDAC8, 1.6%; HDAC9, 4%; HDAC10, 2.4%; HDAC11, 1.9%; Fig. 7A). Analysis showed no significant association for OS and disease free survival between cases with or without HDAC alteration in gastric cancer ($P = .258$ and $P = .510$, respectively; Fig. 7B and C). To explore the potential interactions between HDAC members, a protein–protein interaction network was constructed using STRING database. A total of 11 nodes and 55 edges of 55 were observed in the protein–protein interaction network network (Fig. 7D). In addition, a network for HDACs with the structure or function of neighboring genes constructed using GeneMANIA showed that other 20 genes including DAXX, MEF2A, BRAP, USP39, USP3, USP20, USP51, USP45, USP22, USP33, USP16, USP49, USP44, BCOR, ANKRA2, RBBP4, USP5, USP13, RFXANK, and NRIP1 were closely associated with HDACs (Fig. 7E).

To further explore the biological functions of HDACs, functional enrichment analysis including GO terms (BP: biological process; CC: cellular component; MF: molecular function) and KEGG pathway were conducted using "org.Hs.eg.db," "clusterProfiler," "pathview," "Goplot," "ggplot2" packages. Significantly enriched GO terms and KEGG pathways of HDACs, based on adjusted *P*-values are presented in Figure 8. GO analysis of the top 12 GO terms showed that in molecular function category, these genes were mainly enriched in deacetylase activity, on both nicotinamide adenine dinucleotide-dependent and non-dependent, for histone and non-histone proteins, with H3-K14 having the highest activity (Supplementary Table 1, http://links.lww.com/MD/G226). In biological process group, HDACs were mainly associated with histone H3 deacetylation, histone deacetylation, and protein deacetylation.

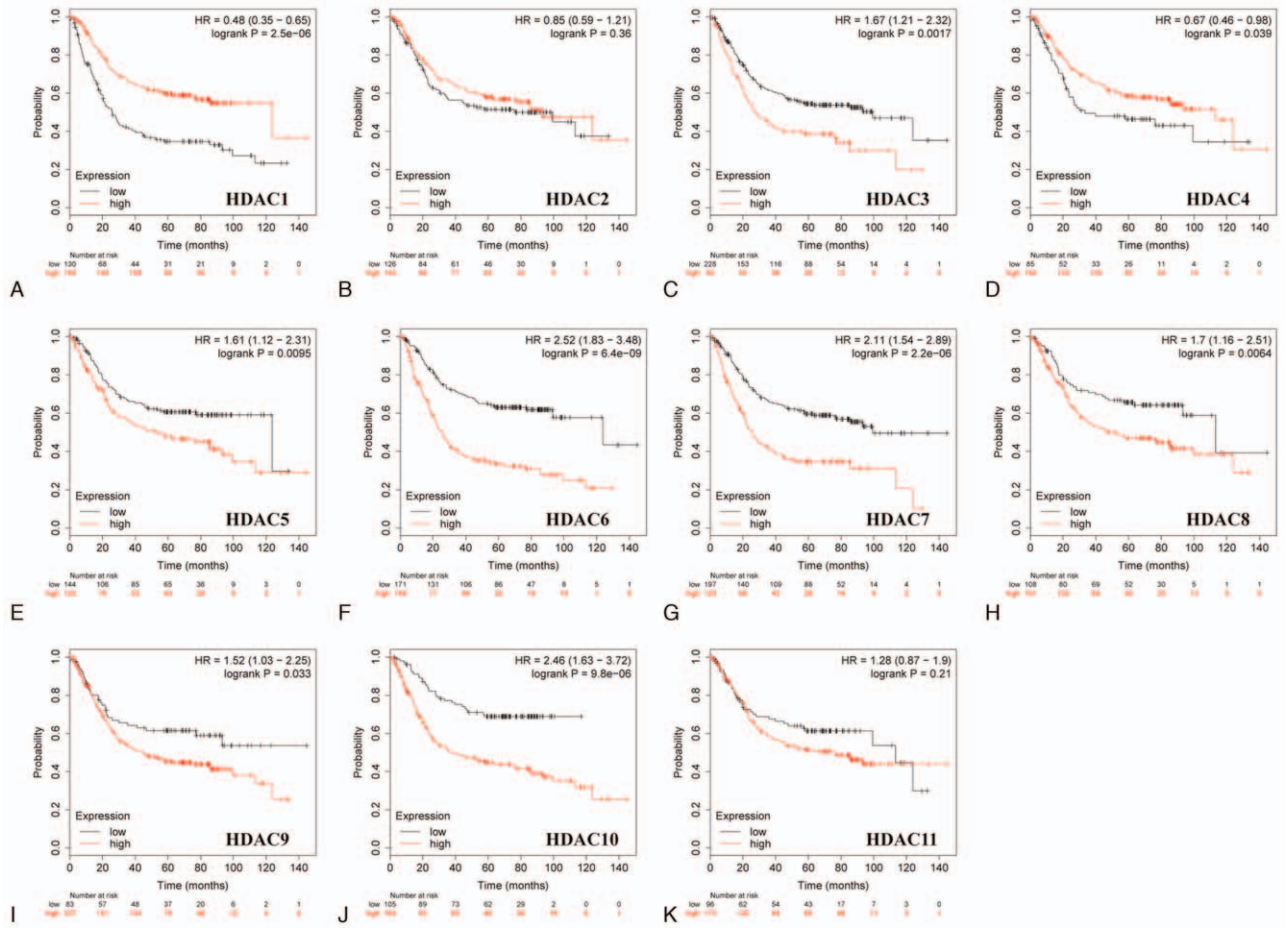

Figure 5. Correlation of HDAC mRNA expression with OS in intestinal gastric cancer patients (Kaplan–Meier plotter). OS = overal survival.

For cellular component term, histone deacetylase complex was the highest enriched cell component. The top 12 enriched KEGG pathway of HDACs showed that HDACs were significantly enriched in 12 pathways, including alcohol metabolism, viral carcinogenesis, thyroid hormone signaling pathway, microRNAs in cancer, notch signaling pathway, longevity regulating pathway-multiple species, amphetamine addiction, chronic myeloid leukemia, cell cycle, apelin signaling pathway, transcriptional misregulation in cancer, and Epstein-Barr virus infection (Supplementary Table 2, http://links.lww.com/MD/G226).

4. Discussion

HDACs are involved in deacetylation of histones and several non-histone proteins including transcription factors and other abundant cellular proteins. They maintain equilibrium between histone acetylation and deacetylation, thus regulating proliferation and cell cycle progression, apoptosis and metastasis.[20–22] Recent studies reported a crucial role of HDACs in carcinogenesis and cancer progression, implying that HDACs are potential targets for cancer therapy. However, only few HDAC family members have been explored in GC. The present study is the first to explore expression levels, prognostic values, genetic alterations, and biological functions of HDACs using bioinformatics analysis.

Aberrant overexpression of HDAC1 is reported in various cancers, and studies report that it is a potential therapeutic target in several tumors.[23–27] Recent studies reported that HDAC1 expression is associated with gastric cancer, where it plays a significant role in distant metastasis and poor patient prognosis.[28,29] A study by Sun et al reported that HDAC1 knockdown repressed the proliferative potential of GC cells and promoted apoptosis induction, implying that HDAC1 is a promising target for gastric cancer therapy.[30] Lin et al reported inhibitory effects of HDAC1-downregulation on metastatic ability in GC cells by targeting the miRNA-34a/CD44 pathway-axis.[27] The findings of the present study showed that HDAC1 was upregulated in GC tissues compared with normal tissues. However, in the current study, high HDAC1 expression was correlated with a good prognosis in GC patients including intestinal and diffuse type, mainly in clinical stage 3 or 4 GC patients, which was not consistent with the previous reports. Therefore, further studies should be conducted to explore the prognostic value of HDAC1 in gastric cancer.

HDAC2, a member of the class I HDACs, is highly upregulated in several cancers.[31] A study by Jung et al reported that inhibition of HDAC2 enhanced expression of p21 (WAF1/Cip1) and repressed expression of cyclin D1, cyclin E2, and CDK2 in cell cycle regulation.[32] Wagner et al reported that high expression of HDAC2 inhibits pro-apoptotic functions of p53,

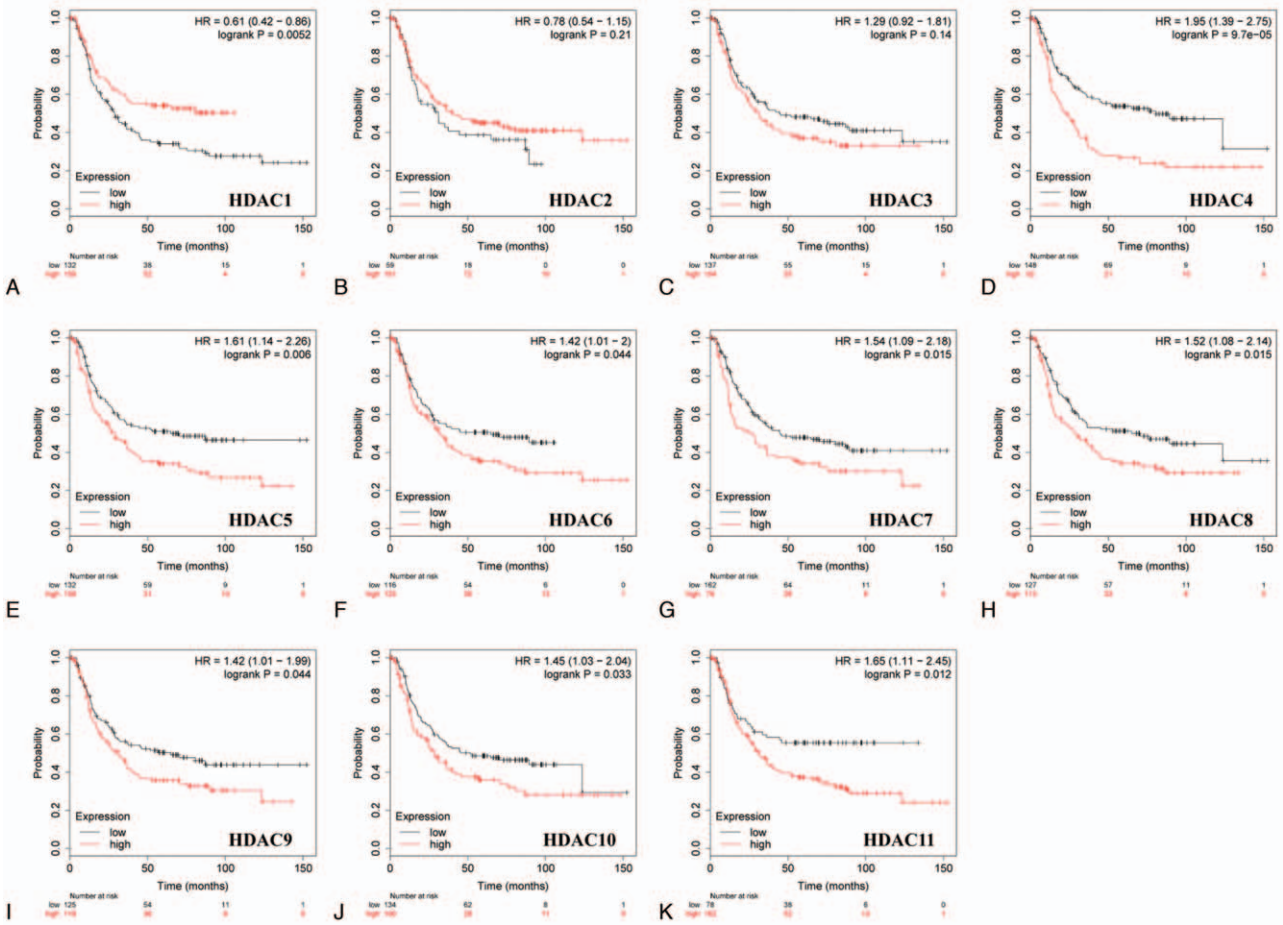

Figure 6. Correlation of HDAC mRNA expression with OS in diffuse gastric cancer patients (Kaplan–Meier plotter). OS = overal survival.

thus inhibiting apoptosis and promoting tumorigenesis.[31] Furthermore, abnormal HDAC2 expression promotes aggressiveness of GC.[33] Moreover, in oral squamous cell carcinoma, HDAC2 increases cell migration and invasion through stabilization of hypoxic induction factor-1α at protein modification level.[34] The findings of the current study showed that expression of HDAC2 was significantly upregulated in GC tissues; however, the expression level was not correlated with overall survival in GC patients. Further studies should be conducted to estimate the prognostic role of HDAC2 in GC.

Previous studies report a negative correlation between expression of HDAC3 and overall survival in some cancer types such as gastric, pancreatic cancer, and glioma.[35–37] In addition HDAC3 is implicated in promoting gastric carcinogenesis through the miR-454-mediated pathway by targeting CHD5.[35] Consistent with findings from previous studies, the findings of the current study showed that low expression of HDAC3 was correlated with favorable OS of clinical stage 1 to 4 GC patients. Analysis showed a significant role of HDAC3 as a candidate biomarker for predicting prognosis of GC.

HDAC4, a key member of class IIa family of HDACs, is highly expressed in a variety of tissues and is associated with cancer progression.[38] Mottet et al performed a study to explore the mechanism of action and reported that HDAC4 is implicated in suppression of p21 (WAF1/Cip1) and has Sp1/Sp3-binding sites in glioblastoma.[39] Jin et al reported that miR-520b inhibits growth of lung cancer cells by targeting HDAC4.[40] Furthermore, HDAC4 promotes cell growth and metastasis by inhibiting expression of p21, p27, E-cadherin, and β catenin, and enhancing expression of CDK2 and vimentin in gastric, glioma, and esophageal carcinoma.[18,41,42] Consistently, the findings of this study showed that HDAC4 was significantly overexpressed in GC tissues, and high expression of HDAC4 was correlated with poor OS in GC patients mainly in clinical stage 4 patients and lymph node positive patients. Therefore, the findings of this study imply that HDAC4 can be used as a diagnostic or prognostic indicator in GC.

Studies on HDAC5 and HDAC7 in GC are limited. Liu et al carried out a study on non-small cell lung cancer, and reported an important role of miR-589-5p/HDAC5 pathway-axis in growth and metastasis of tumor cells, implying that HDAC5 is a potential oncogene.[43] HDAC7 modulates cell cycle progression by regulation of c-Myc expression.[44] Furthermore, Yu et al reported that HDAC7 expression was negatively correlated with overall survival in patients with GC.[45] The findings of the current study showed that elevated HDAC5/7 expression was correlated with poor prognosis in GC including intestinal and diffuse type, mainly those classified as clinical stage 2/3/4 and lymph node positive patients. However, no significant differences

Table 1

Correlation of HDAC mRNA expression with clinical stages of GC patients.

HDACs	Clinical stages	Cases	HR (95%CI)	*P* value
HDAC1	1	69	0.37 (0.12–1.15)	.074
	2	145	0.72 (0.37–1.38)	.32
	3	319	0.5 (0.35–0.69)	.000032*
	4	152	0.64 (0.43–0.94)	.023*
HDAC2	1	69	0.35 (0.12–1.08)	.057
	2	145	1.79 (0.96–3.33)	.062
	3	319	1.25 (0.86–1.83)	.24
	4	152	0.81 (0.54–1.2)	.3
HDAC3	1	69	2.89 (1.08–7.73)	.027*
	2	145	3.29 (1.29–8.35)	.0081*
	3	319	1.54 (1.16–2.05)	.0029*
	4	152	0.65 (0.43–0.99)	.044*
HDAC4	1	69	1.95 (0.59–6.38)	.26
	2	145	1.6 (0.85–2.99)	.14
	3	319	0.69 (0.46–1.04)	.075
	4	152	1.66 (1.11–2.47)	.013*
HDAC5	1	69	0.45 (0.13–1.57)	.2
	2	145	2.12 (1.14–3.94)	.015*
	3	319	2.08 (1.39–3.12)	.00026*
	4	152	1.62 (1.09–2.41)	.016*
HDAC6	1	69	4.75 (1.72–13.08)	.00089*
	2	145	2.08 (1.13–3.82)	.016*
	3	319	2.29 (1.57–3.34)	.0000098*
	4	152	1.37 (0.93–2.01)	.11
HDAC7	1	69	0.56 (0.2–1.56)	.26
	2	145	2.06 (1.12–3.77)	.018*
	3	319	1.68 (1.26–2.23)	.00034*
	4	152	1.58 (1.07–2.32)	.019*
HDAC8	1	69	1.61 (0.54–4.83)	.39
	2	145	2.09 (1.12–3.92)	.018*
	3	319	1.71 (1.16–2.51)	.006*
	4	152	1.36 (0.91–2.03)	.14
HDAC9	1	69	0.54 (0.17–1.73)	.3
	2	145	2.06 (1.02–4.18)	.041*
	3	319	0.79 (0.59–1.05)	.1
	4	152	1.54 (1.05–2.26)	.026*
HDAC10	1	69	2 (0.59–6.74)	.26
	2	145	1.73 (0.84–3.54)	.13
	3	319	1.82 (1.25–2.65)	.0014*
	4	152	1.59 (1.05–2.42)	.029*
HDAC11	1	69	4.11 (0.53–31.66)	.14
	2	145	1.88 (0.92–3.86)	.079
	3	319	1.83 (1.23–2.71)	.0023*
	4	152	0.73 (0.47–1.13)	.15

GC = gastric cancer; HDACs = histone deacetylases; HR = hazard ratio.
* *P* value < .05.

Table 2

Correlation of HDAC mRNA expression with lymph node status of GC patients.

HDACs	Lymph node status	Cases	HR (95%CI)	*P* value
HDAC1	Negative	76	0.4 (0.17–0.92)	.025*
	Positive	437	0.54 (0.41–0.7)	.0000028*
HDAC2	Negative	76	2.44 (0.72–8.28)	.14
	Positive	437	0.84 (0.64–1.1)	.12
HDAC3	Negative	76	1.49 (0.66–3.4)	.34
	Positive	437	1.2 (0.92–1.56)	.18
HDAC4	Negative	76	1.9 (0.82–4.38)	.13
	Positive	437	1.79 (1.36–2.36)	.000026*
HDAC5	Negative	76	0.54 (0.22–1.32)	.17
	Positive	437	1.74 (1.34–2.27)	.000026*
HDAC6	Negative	76	2.93 (1.26–6.85)	.0094*
	Positive	437	1.83 (1.41–2.38)	.0000051*
HDAC7	Negative	76	1.77 (0.69–4.49)	.23
	Positive	437	1.55 (1.18–2.04)	.0014*
HDAC8	Negative	76	1.84 (0.8–4.21)	.14
	Positive	437	1.58 (1.2–2.06)	.00084*
HDAC9	Negative	76	3.56 (1.39–9.12)	.0049*
	Positive	437	1.28 (0.95–1.71)	.1
HDAC10	Negative	76	1.85 (0.62–5.54)	.26
	Positive	437	1.91 (1.45–2.52)	.0000028*
HDAC11	Negative	76	2.08 (0.91–4.73)	.075
	Positive	437	1.4 (1.05–1.86)	.02*

GC = gastric cancer; HDACs = histone deacetylases; HR = hazard ratio.
* *P* value < .05.

were observed for expression levels of HDAC5/7 between GC and normal tissues.

Aberrant expression of HDAC6 has been reported in several diseases, such as neurodegenerative diseases, cardiovascular disease, and cancer.[46,47] Previous studies reported that HDAC6 is implicated in cancer initiation and progression by modulating cell proliferation, angiogenesis, motility, invasion, and metatasis of tumor cells.[48] Li et al explored the mechanism of action of HDAC6 and reported that targeting of non-histone proteins such as α-tubulin, cortactin, and heat shock protein 90 by HDAC6 contributed to tumorigenesis and cancer progression. Moreover, HDAC6 affected immune system by regulating program death receptor-1 and program death receptor ligand-1 receptor.[49] Aberrant expression of HDAC6 has been reported in GC samples, and studies report that it is an oncogene.[50,51] In the present study, HDAC6 was downregulated in GC tissues, and patients with low HDAC6 expression level showed longer OS including intestinal and diffuse type, clinical stage 1/2/3, lymph node positive/negative patients. These findings imply that HDAC6 is a potential prognostic biomarker or therapeutic target in gastric cancer.

Numerous studies report aberrant overexpression of HDAC8 in a variety of cancers, including gastric, liver, breast, and oral squamous cell carcinoma.[52–56] For example, HDAC8 was reported as an oncogene that promotes malignant progression of breast cancer. Menbari et al reported that HDAC8 may exert its activity by protecting Notch1 from Fbwx7-facilitated protein degradation, resulting in activation of breast cancer stem cells.[52,53] A study on HCC reported that knockdown of HDAC8 inhibited tumor growth and induced apoptosis by upregulating expression of p53 and acetylation of p53 at Lys382.[54] In addition, a study by Song reported that HDAC8 plays a role in promoting tumorigenesis in GC.[55] Consistent with the findings from these studies, the findings from the current study exhibited that HDAC8 was predominantly overexpressed in GC tissues, and it was significantly correlated with poor prognosis in patients with GC mainly in clinical stage 2/3 and lymph node positive patients. Therefore, HDAC8 is a potential target for anti-GC therapeutics.

HDAC9 plays a critical role in progression of tumor. Xiong et al reported a negative correlation between expression of HDAC9 and OS in GC patients[12]; downregulation of miRNA-383-5p was correlated with poor patient survival and metastasis in GC by targeting HDAC9.[57] In addition, targeting of HDAC9 by miR-377 promoted cell migration and enhanced the proliferative potential of oral squamous cell carcinoma.[58]

Table 3

Correlation of HDAC mRNA expression with treatment of GC patients.

HDACs	Treatment	Cases	HR (95%CI)	*P* value
HDAC1	Surgery alone	393	0.69 (0.51–0.93)	.013*
	5 FU based adjuvant	157	2.03 (1.36–3.05)	.00048*
HDAC2	Surgery alone	393	0.88 (0.66–1.18)	.39
	5 FU based adjuvant	157	0.39 (0.15–1.01)	.046*
HDAC3	Surgery alone	393	0.83 (0.62–1.12)	.22
	5 FU based adjuvant	157	1.65 (1.16–2.35)	.0052*
HDAC4	Surgery alone	393	1.59 (1.19–2.12)	.0016*
	5 FU based adjuvant	157	1.9 (0.74–4.86)	.17
HDAC5	Surgery alone	393	1.54 (1.15–2.05)	.0032*
	5 FU based adjuvant	157	1.61 (0.63–4.1)	.31
HDAC6	Surgery alone	393	1.36 (0.98–1.87)	.062
	5 FU based adjuvant	157	1.63 (1.13–2.36)	.009*
HDAC7	Surgery alone	393	1.5 (1.09–2.04)	.011*
	5 FU based adjuvant	157	1.39 (0.98–1.97)	.062
HDAC8	Surgery alone	393	1.43 (1.07–1.92)	.016*
	5 FU based adjuvant	157	2.08 (0.78–5.5)	.13
HDAC9	Surgery alone	393	1.51 (1.1–2.08)	.011*
	5 FU based adjuvant	157	0.64 (0.45–0.91)	.012*
HDAC10	Surgery alone	393	1.36 (0.97–1.9)	.074
	5 FU based adjuvant	157	3.43 (0.78–15)	.082
HDAC11	Surgery alone	393	1.36 (0.99–1.85)	.054
	5 FU based adjuvant	157	3.05 (1.06–8.79)	.031*

5 FU = 5 fluorouracil; GC = gastric cancer; HDACs = histone deacetylases; HR = hazard ratio.
* *P* value < .05.

Consistent with findings from previous studies, analysis in the current study showed that HDAC9 was highly expressed in GC tissues, and expression level of HDAC9 varied significantly in different pathological stages. Furthermore, high HDAC9 expression level was correlated with poor prognosis in intestinal and diffuse type GC patients, as well as clinical stage 2/4 and lymph node negative patients.

Only a few studies have explored the roles of HDAC10 or HDAC11 in tumorigenesis and cancer progression. Jin et al reported that HDAC10 was downregulated in GC tissues, which

Table 4

Correlation of HDAC mRNA expression with HER2 status of GC patients.

HDACs	HER2 status	Cases	HR (95%CI)	*P* value
HDAC1	Negative	641	0.56 (0.43–0.74)	.00002*
	Positive	424	0.82 (0.62–1.09)	.17
HDAC2	Negative	641	0.82 (0.63–1.07)	.14
	Positive	424	1.36 (0.87–2.14)	.18
HDAC3	Negative	641	1.37 (1.09–1.72)	.0064*
	Positive	424	1.43 (1.08–1.89)	.011*
HDAC4	Negative	641	1.86 (1.42–2.43)	.0000036*
	Positive	424	0.8 (0.53–1.21)	.29
HDAC5	Negative	641	1.63 (1.25–2.12)	.00027*
	Positive	424	1.43 (0.98–2.09)	.064
HDAC6	Negative	641	2.1 (1.64–2.67)	.0000000012*
	Positive	424	1.88 (1.36–2.6)	.00011*
HDAC7	Negative	641	1.69 (1.34–2.13)	.0000061*
	Positive	424	1.83 (1.38–2.42)	.000017*
HDAC8	Negative	641	1.38 (1.05–1.8)	.019*
	Positive	424	1.74 (1.18–2.56)	.0049*
HDAC9	Negative	641	1.33 (1.04–1.7)	.023*
	Positive	424	1.2 (0.89–1.62)	.23
HDAC10	Negative	641	1.66 (1.25–2.2)	.00034*
	Positive	424	1.79 (1.16–2.77)	.0076*
HDAC11	Negative	641	1.38 (1.03–1.84)	.03*
	Positive	424	1.42 (0.98–2.06)	.063

CI = confidence intervals; GC = gastric cancer; HDACs = histone deacetylases; HER2 = human epidermal growth factor receptor 2; HR = hazard ratio.
* *P* value < .05.

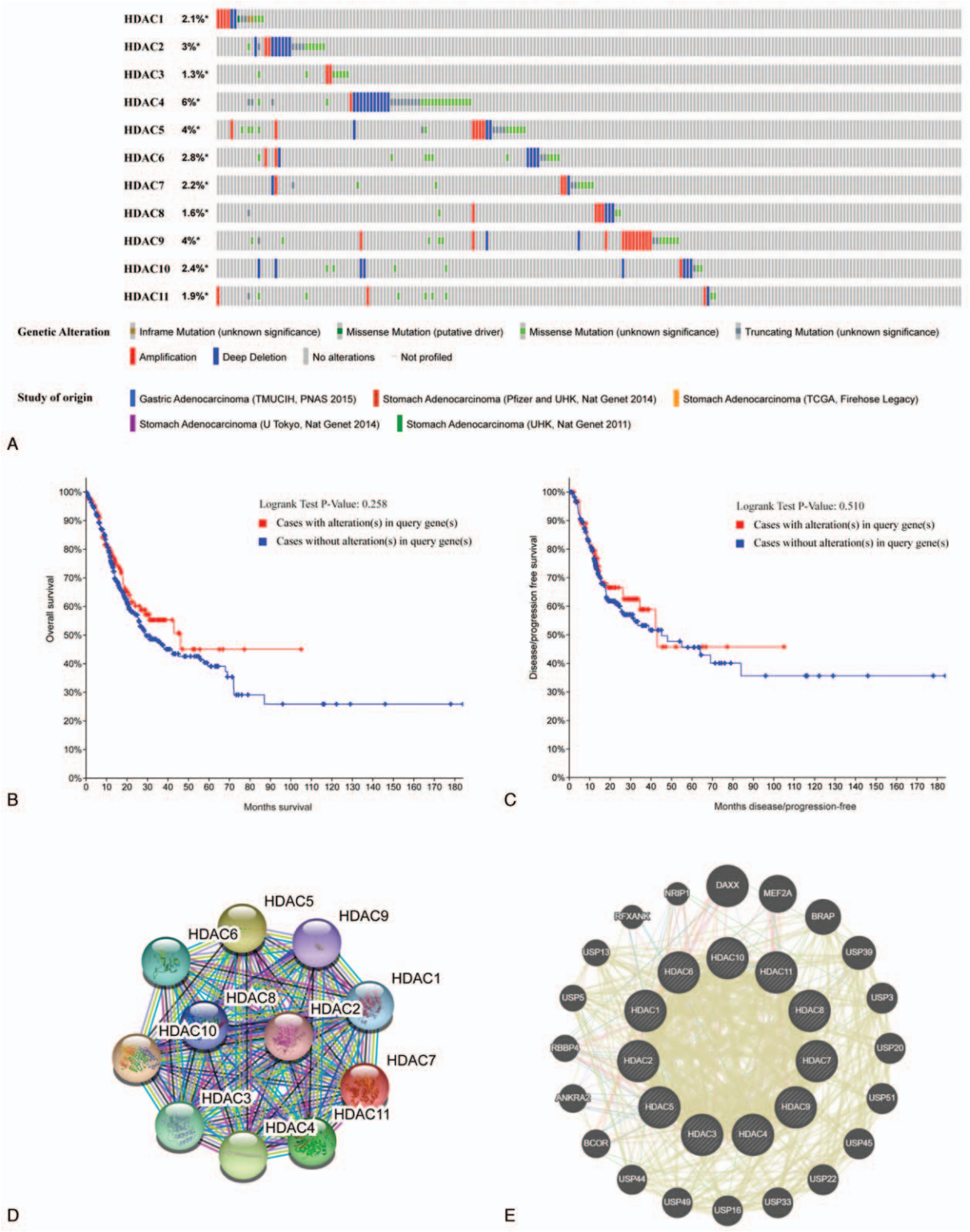

Figure 7. Alterations and network of HDACs (cBioPortal, String, and GeneMANIA). HDACs = histone deacetylases.

was correlated with poor prognosis in GC patients.[59] This was consistent with findings of the present study that expression of HDAC10 was downregulated in GC tissues; however, low expression of HDAC10 was correlated with good OS, which was not consistent with previous reports. Low HDAC11 expression level was correlated with favorable OS in GC patients mainly for the diffuse type. However, differential HDAC11 expression was not observed between GC and normal tissues. Further studies should be conducted to explore the roles of HDAC10/HDAC11 in gastric cancer.

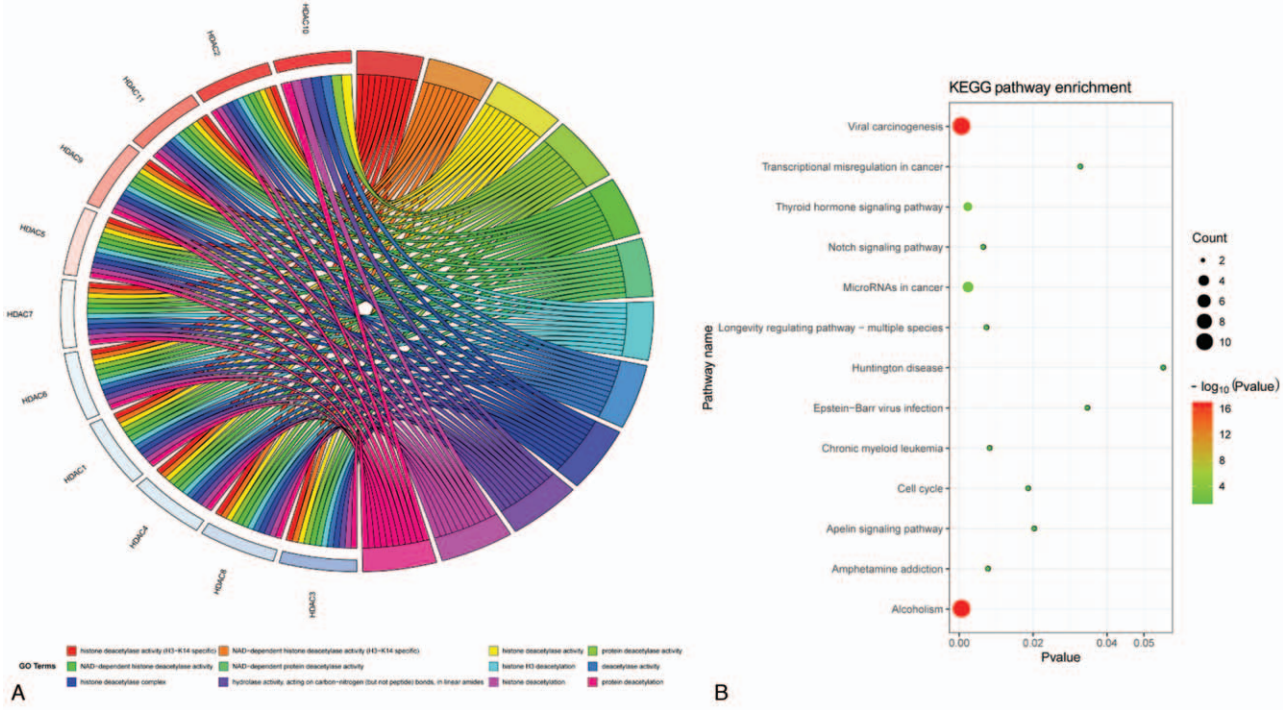

Figure 8. The enrichment analysis of HDACs (R software). HDACs = histone deacetylases.

As promising tumor suppressors or oncogenes, genetic alterations of HDACs may be correlated with carcinogenesis, progression, and prognosis of GC. Relatively low consistent levels of alterations were observed in each HDAC in GC, which had no impact on OS or disease free survival. Previous studies report that HDAC alterations may not affect GC prognosis. To further explore the biological functions of HDACs, network analysis was performed for HDAC family members. The genes were mainly enriched in deacetylase activity, tumor-related pathways, and growth-related pathways such as microRNAs in cancer, notch signaling pathway, longevity regulating pathway-multiple species, cell cycle, transcriptional misregulation in cancer. The findings of this study show the potential role of HDACs as therapeutic targets in gastric cancer.

In view of the important roles of HDACs in a variety of biological processes in carcinogenesis and cancer progression, a number of clinical trials of HDAC inhibitors (HDACis) have been carried out. To date, 4 HDACis including vorinostat (SAHA), romidepsin (FK228), panobinostat ((LBH589), and belinostat (PXD101) have been approved by the US Food and Drug Administration in the treatment of several hematological malignancies and lymphomas,[60–63] while numerous clinical trials are ongoing for advanced or refractory tumors. For instance, San-Miguel et al reported a phase III trial of the pan-HDACi panobinostat in combination with bortezomib and dexamethasone and schedules in 768 patients with relapsed multiple myeloma; the median OS of panobinostat group was 40.3 months while that of placebo group was 35.8 months.[64] Similar encouraging results have been reported on a phase I to II clinical trial of the cyclic peptide HDACi romidepsin combined with dexamethasone and bortezomib.[65] Similarly, a phase III trial found that, compared with the placebo-controlled group, the subtype-selective HDACi tucidinostat (also named chidamide) plus exemestane regimen could improve progression-free survival for postmenopausal patients with advanced, hormone receptor-positive breast cancer, which may serve as a new treatment option.[66] The results of current trial revealed that HDAC inhibitors could be a promising avenue for cancer treatment; however, studies on HDACis in gastric cancer therapy are limited, which requires further research.

5. Conclusion

In summary, expression of HDAC2/4 was significantly upregulated in GC, and aberrant expression of HDAC1/3/4/5/6/7/8/10/11 was associated with prognosis in GC. Moreover, expression levels of the HDACs were correlated with different Lauren classifications, and clinical stages, lymph node status, treatment, and HER2 status. The findings of this study show the role of HDACs as potential diagnostic or prognostic biomarkers and therapeutic targets in GC. However, further research is required to validate these findings.

Author contributions

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