Clinical significance of HDAC1, -2 and -3 expression levels in esophageal squamous cell carcinoma

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Abstract. The present study analyzed the expression of the histone deacetylase (HDAC) 1, 2 and 3 in primary esophageal squamous cell carcinoma (ESCC) samples and how their levels correlate with clinicopathological parameters. ESCC patients (n=88) in the present study had received no previous treatment before undergoing surgical excision. The mRNA expression of *HDAC1*, *-2* and *-3* were detected by semi-quantified PCR in ESCC samples and distal normal samples. The relationship of HDAC1, -2 and -3 expression with clinicopathological parameters was analyzed by χ^2 test. The correlation among these HDACs was analyzed by Pearson's correlation test. Compared with distal normal tissues, ESCC samples had higher expression of HDAC1, but not HDAC2 or HDAC3 (P<0.05). The expression of HDACs was different between Kazak and Han ethnicities. The expression of HDAC2 was correlated with invasion depth (P<0.05), but not with sex, age, metastasis, or the degree of tumor differentiation (P>0.05). There was no association between HDAC1 or HDAC3 and clinicopathological parameters (P>0.05). For the Kazak and Han ethnicities, HDAC1 expression was present in male patients, patients with well/moderate differentiated ESCC and T3 and T4 ESCC (P<0.01). HDAC1 in patients aged <60 was associated with ethnicity (P<0.05). HDAC2 expression was different in positive LN metastasis, well/moderate differentiation and T3 and T4 ESCC (P<0.01). HDAC3 expression in male patients, patients with negative LN metastasis and well/moderate differentiation ESCC was associated with ethnicity (P<0.05). Additionally, the expression levels of HDAC1, -2 and -3 did not correlate with each other. Thus, HDAC1 expression may be used as a risk factor for ESCC and HDAC2 levels may be used to predict invasion depth. The expression of HDAC1, -2 and -3 has ethnic differences.

Introduction

Esophageal cancer is the sixth leading cause of cancer-related mortality. It caused the mortality of 440,000 people in 2013 (1). It is the third deadliest cancer in Chinese males and the fifth deadliest malignance in Chinese females (2). In China and other Asian countries, 95% of the esophageal cancer is esophageal squamous cell carcinoma (ESCC) and the 5-year overall survival rate is ~10-20% (3). Therefore, it is important to identify the tumor-specific markers in ESCC (4).

Histone deacetylases (HDACs) are expressed in plants, animals, fungi, archaebacteria and eubacteria (5). HDACs are classified into Class I, II and IV or Class III (coenzyme-nicotinamide adenine dinucleotide). HDAC1, -2, -3 and -8 belong to Class I (6). HDACs together with histone acetylases can regulate gene transcription and carcinogenesis by modulating chromatin structure (7). Class I HDACs have been shown to be widely expressed in all kinds of solid cancers (8,9). HDAC1 expression in ESCC is higher compared with normal esophageal mucosa. When cancer cells invade the deep layer of the esophageal wall, the expression of HDAC 1 is significantly decreased compared with normal control samples (10). Nevertheless, the expression and role of HDAC2 and 3 in ESCC remain to be elucidated.

The relationship between histone deacetylation and cellular events, including cell proliferation, differentiation and cell cycle regulation has been demonstrated (11-13). For example, HDACs may inhibit target gene expression by binding to the transcriptional cofactor PC3/Tis21, thereby inhibiting cell proliferation (14). HDAC2 overexpression enhances the aggressiveness of gastric carcinoma cells and HDAC2 inhibition attenuates the carcinogenic potential of gastric carcinoma cells in xenotransplanted nude mice (13).

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Abbreviations: ESCC, esophageal squamous cell carcinoma; HDAC, histone deacetylase

In addition, several studies have shown that HDAC3 is over-expressed in various solid tumors and is closely associated with poor prognosis (14-16).

The present study investigated the expression levels of HDAC1, -2 and -3 and their clinical significance in ESCC. The association of HDAC1, -2 and -3 with clinicopathological characteristics of ESCC was also analyzed.

Materials and methods

Patient information and tissue specimens. Primary ESCC tissues and paired distal normal tissues (>5 cm away from the margin of tumor tissue) were collected from 88 patients (Kazak, n=40; Han, n=44). Esophagus excision was performed between December 2009 and September 2015 at the Xinjiang Medical University Affiliated Cancer Hospital. No patient received preoperative chemotherapy or radiotherapy. ESCC diagnosis was confirmed by pathology. Patient characteristics including sex, age, lymph node (LN) metastasis, differentiation and depth of invasion were recorded (Table I). The present study research was approved by the Ethics Committee of Xinjiang Medical University and all patients provided written informed consent.

Reverse transcription semi-quantified PCR. Total RNA was extracted with TRIzol (Invitrogen, Thermo Fisher Scientific, Inc.) from ESCC tissues and paired distal normal tissues. Reverse transcription of RNA was performed with avian myeloblastosis virus reverse transcriptase (Promega Corporation). Reverse transcription was performed under the following heat conditions: 55°C for 90 min and 4°C for 10 min. The cDNA products were identified on a 2% agarose gel with ethidium bromide. The PCR reaction system included 2 μl reverse-transcribed cDNA, 10 μl 2XPCR master mix, 1 μl of each primer (10 μM) and 6 μl ddH$_2$O (Ready-to-Use PCR kit, Beijing Transgen Biotech Co., Ltd.). Amplification was performed on an iCycler™ Thermal Cycler (Bio-Rad Laboratories, Inc.). GAPDH was an internal control.

The PCR primers were: *HDAC1* forward, 5'-AGTGCG GTGGTCTTACAGTG-3', *HDAC1* reverse, 5'-TCTCCCTCC TCTTCAGAATCG-3', *HDAC2* forward, 5'-GCTGGTCTT GAACTCCTT-3', *HDAC2* reverse, 5'-TACAACCCATCT GGCATC-3', *HDAC3* forward, 5'-GGGACATTATTGGCA GTG-3', *HDAC3* reverse, 5'-GGATTCAGGTGTTAGGGA G-3', *GAPDH* forward, 5'-GCGGGCTCTCCAGAACAT CAT-3' and *GAPDH* reverse, 5'-CCAGCCCCAGCGTCA AAGGTG-3'. The thermocyling conditions were as follows: Initial denaturation at 95°C for 5 min; 35 cycles of 95°C for 30 sec, 58°C for 20 sec for *HDAC1*. 54°C for 20 sec for *HDAC2*, 58°C for 30 sec for *HDAC3* and 60°C for 30 sec for *GAPDH*, 72°C for 30s; and a final extension at 72°C for 7 min. The PCR products were identified on a 1.5% agarose gel with ethidium bromide and analyzed with the Gel Doc XR System (Bio-Rad Laboratories, Inc.). The DNA ladder maker was purchased from Sangon Biotech Co., Ltd. The intensities of PCR product bands were quantified using Quantity One software version 4.5.2 (Bio-Rad Laboratories, Inc.). The quantity of each PCR product band was standardized to that of *GAPDH*. The presence or absence of bands was considered positive or negative. Grey scale ratio >2 was defined as high expression.

Table I. Clinicopathological characteristics of esophageal squamous cell carcinoma patients.

Basic data	Number
Sex	
Male	62
Female	26
Ethnicity	48
Han	40
Kazakh	
Age	
<60 yrs	46
≥60 yrs	42
LN Metastasis	
Positive	46
Negative	42
Differentiation	
Well and Moderate	69
Poor	19
Depth of invasion	
T1 and T2	13
T3 and T4	75

ESCC, esophageal squamous cell carcinoma; LN, lymph node metastases; T1, tumors only invade the lamina propria and submucosa; T2, tumors invade the muscular layer; T3, tumors invade the esophageal fibrous membrane; T4, tumors invade adjacent organs.

Statistical analysis. SPSS 17.0 software (SPSS Inc.) was used for data analysis. Experiments were performed in triplicate. Measurement data were presented as the mean ± SD. Count data were expressed as the number (%). Correlations among HDAC1, -2 and -3 expression were analyzed by the Pearson correlation test. Differences in gene expression were analyzed using a paired t-test and variances in clinicopathologic features were analyzed using χ^2 test. P<0.05 was considered to indicate a statistically significant difference.

Results

Patient characteristics. First, the basic information of the patients was investigated (Table I). Of the 88 patients, 26 (29.5%) were women and 62 (70.5%) were men. Their mean age was 58.2 years, 37-84 years old. The most common tumor location was at the middle and lower esophagus (95.5%). There were 69 ESCC cases with moderate to high differentiation and 19 with poor differentiation. A total of 46 cases had LN metastasis and 42 did not have LN metastasis. There were 13 cases of T_1+T_2 and 75 cases of T_3+T_4.

Expression levels of HDAC1, -2 and -3 in human ESCC and distal normal tissues. To compare the expression levels of *HDAC1, -2* and *-3* in tumor issues and distal normal tissues, reverse transcription semi-quantified PCR analysis was performed. The representative amplification results of *HDAC1, -2* and *-3* are presented in Fig. 1A. It demonstrated

Table II. Expression of *HDAC1*, *-2* and *-3* mRNA in tumor and normal tissue.

Expression of mRNA	Specimens	No	Positive (+)	Negative (-)	Positive rate (%)	X^2	P-value
HDAC1	T	88	43	45	48.9 (43/88)	5.312	0.021[a]
	N	88	28	60	31.8 (28/88)		
HDAC2	T	88	68	20	77.3 (68/88)	1.828	0.176
	N	88	75	13	85.2 (75/88)		
HDAC3	T	88	82	6	93.2 (82/88)	1.628	0.202
	N	88	77	11	87.5 (77/88)		

[a]P<0.05. T, Tumor tissues; N, Normal tissues; HDAC, histone deacetylase.

Table III. Expression of *HDAC1*, *HDAC2* and *HDAC3* mRNA in tumor and normal tissue of Han and Kazak people.

Expression of mRNA	Specimens		Positive no. (+)		Negative no. (-)			X^2	P-value
HDAC1	T	43	Han	31	45	Han	17	10.443	0.001[a]
			Kazak	12		Kazak	28		
	N	28	Han	21	60	Han	27	6.930	0.008[a]
			Kazak	7		Kazak	33		
HDAC2	T	68	Han	44	20	Han	4	12.458	0.176
			Kazak	24		Kazak	16		
	N	75	Han	46	13	Han	3	6.571	0.010[a]
			Kazak	29		Kazak	10		
HDAC3	T	82	Han	48	6	Han	0	7.727	0.005[a]
			Kazak	34		Kazak	6		
	N	77	Han	45	11	Han	3	3.771	0.052
			Kazak	32		Kazak	8		

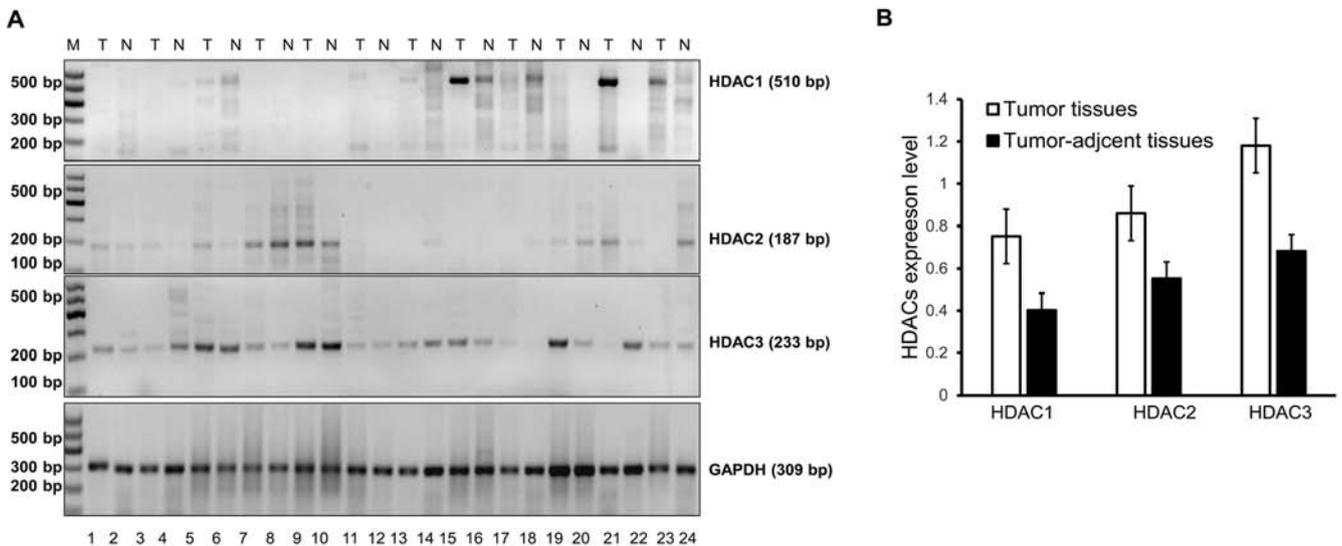[a]P<0.05. T, Tumor tissues; N, Normal tissues; HDAC, histone deacetylase.

Figure 1. Expression of *HDAC1*, *-2* and *-3* mRNA. *HDAC1*, *-2* and *-3* mRNA levels in ESCC samples and normal tissues were analyzed by semi-quantified PCR. (A) Representative amplification results of *HDAC1*, *-2* and *-3* in patient specimens. (B) Normalized *HDAC1*, *-2* and *-3* expression level in human ESCC and normal tissues. T, primary tumor; N, paired normal tissues; M, marker; ESCC, esophageal squamous cell carcinoma; HDAC, histone deacetylase.

Table IV. Correlation of *HDAC1* expression with clinicopathologic characteristics of esophageal squamous cell carcinoma patients (n=88).

Clinicopathological characteristics	No.	Expression of *HDAC1* mRNA			X^2	P-value
		Positive (+)	Negative (-)	Positive rate (%)		
Sex						
Male	62	31	31	50.0 (31/62)	0.108	0.742
Female	26	12	14	46.2 (12/26)		
Age						
<60 yrs	46	20	26	43.5 (20/46)	1.119	0.290
≥60 yrs	42	23	19	54.8 (23/42)		
LN Metastasis						
Positive	46	22	24	47.8 (22/46)	0.042	0.839
Negative	42	21	21	50.0 (21/42)		
Differentiation						
Well and Moderate	69	36	33	52.26 (36/69)	1.401	0.236
Poor	19	7	12	36.8 (7/19)		
Depth of invasion						
T1 and T2	13	6	7	46.2 (6/13)	0.045	0.832
T3 and T4	75	37	38	49.3 (37/75)		

LN, lymph node metastases; T1, tumors only invade the lamina propria and submucosa; T2, tumors invade the muscular layer; T3, tumors invade the esophageal fibrous membrane; T4, tumors invade adjacent organs; HDAC, histone deacetylase.

Table V. Correlation of *HDAC2* expression with clinicopathologic characteristics of esophageal squamous cell carcinoma patients (n=88).

Clinicopathological characteristics	No.	Expression of *HDAC2* mRNA			X^2	P-value
		Positive (+)	Negative (-)	Positive rate (%)		
Sex						
Male	62	47	15	75.8 (47/62)	0.257	0.612
Female	26	21	5	80.8 (21/26)		
Age						
<60 yrs	46	37	9	80.4 (37/46)	0.549	0.459
≥60 yrs	42	31	11	73.8 (31/42)		
LN Metastasis						
Positive	46	39	7	84.8 (39/46)	3.095	0.079
Negative	42	29	13	69.0 (29/42)		
Differentiation						
Well and Moderate	69	52	17	75.4 (52/69)	0.664	0.415
Poor	19	16	3	84.2 (16/19)		
Depth of invasion						
T1 and T2	13	6	7	13	8.411	0.004[a]
T3 and T4	75	62	13	75		

[a]P<0.05. LN, lymph node metastases; T1, tumors only invade the lamina propria and submucosa; T2, tumors invade the muscular layer; T3, tumors invade the esophageal fibrous membrane; T4, tumors invade adjacent organs; HDAC, histone deacetylase.

that *HDAC1* was 510 bp in length, *HDAC2* was 187 bp, *HDAC3* was 233 bp and *GAPDH* was 309 bp (Fig. 1A).

Following quantification, expression of *HDAC1*, *-2* and *-3* mRNA was elevated in tumor tissues compared with

Table VI. Correlation of *HDAC3* expression with clinicopathologic characteristics of esophageal squamous cell carcinoma patients (n=88).

| Clinicopathological characteristics | No. | Expression of *HDAC3* mRNA | | | X^2 | P-value |
		Positive (+)	Negative (-)	Positive rate (%)		
Sex						
Male	62	59	3	95.2 (59/62)	1.294	0.255
Female	26	23	3	88.5 (23/26)		
Age						
<60 yrs	46	45	1	97.8 (45/46)	3.272	0.070
≥60 yrs	42	37	5	88.1 (37/42)		
LN Metastasis						
Positive	46	43	3	93.5 (43/46)	0.013	0.908
Negative	42	39	3	92.9 (39/42)		
Differentiation						
Well and Moderate	69	64	5	92.8 (64/69)	0.092	0.761
Poor	19	18	1	94.7 (18/19)		
Depth of invasion						
T1 and T2	13	13	0	100 (13/13)	1.116	0.291
T3 and T4	75	69	6	92.0 (69/75)		

LN, lymph node metastases; T1, tumors only invade the lamina propria and submucosa; T2, tumors invade the muscular layer; T3, tumors invade the esophageal fibrous membrane; T4, tumors invade adjacent organs; HDAC, histone deacetylase.

distal normal tissue, although no significant difference was observed (Fig. 1B). The *HDAC1* mRNAs were positive in 48.9% (43/88) of the tumor tissues, *HDAC2* mRNAs were positive in 77.3 (68/88) of the tumor tissues and *HDAC3* mRNAs were positive in 93.2% (82/88) of the tumor tissues. Their positive rate in distal normal tissues was 31.8% (28/88), 85.2% (75/88) and 87.5% (77/88), respectively. The expression of *HDAC1*, but not *HDAC2* or *HDAC3*, was higher in ESCC samples than in distal normal samples (P<0.05; Table II). The expression of *HDAC1*, *-2* and *-3* in patients of different ethnicities was also analyzed. As shown in Table III, the expression of *HDAC1* in tumor tissue and normal tissue of Kazak people was lower compared with Han people (P<0.05). The expression of *HDAC2* in normal tissue of Kazak people was decreased compared with that in Han people. The expression of *HDAC3* in tumor tissue of Kazak people was decreased compared with that in Han people (P<0.05).

Relationship of HDAC1, -2 and -3 expression with clinicopathological characteristics. Next the relationship of HDAC1, -2 and -3 expression with the clinicopathological characteristics of ESCC patients was detected. No significant differences were found in correlation analysis between HDAC1 expression and clinicopathological indexes (Table IV). The expression of HDAC2 was significantly related with invasion depth (P<0.05; Table V). However, there were no significant correlation between HDAC2 and age, sex, depth of invasion and tumor differentiation. Moreover, no significant differences were found between HDAC3 expression and clinicopathological indexes

(Table VI). For the Kazak and Han ethnicities, the expression of HDAC1 in male patients, patients with well and moderate differentiated ESCC and T3 and T4 ESCC were significantly related with ethnicity (P<0.01; Table VII). The expression of HDAC1 in patients less than 60 years old was related with ethnicity (P<0.05; Table VII). The expression of HDAC2 in positive LN metastasis, well and moderate differentiation and T3 and T4 stages were significantly related with ethnicity (P<0.01; Table VIII). The expression of HDAC2 in female patients and negative LN metastasis were related with ethnicity (P<0.05; Table VIII). The expression of HDAC3 in male, negative LN metastasis and well and moderate differentiation was related with ethnicity (P<0.05; Table IX).

Correlation among HDAC1, -2 and -3 expression in human ESCC tissues. The correlation among HDAC1, -2 and -3 was obtained by using Pearson's correlation test. The result showed that there were no significant correlations between HDAC1 and HDAC2 (r=-0.042, P=0.694), HDAC1 and HDAC3 (r=-0.084, P=0.430), or HDAC2 and HDAC3 (r=-0.176, P=0.099) in ESCC tissues (Fig. 2). These results indicated that there was no correlation among the three HDACs.

Discussion

ESCC is a common malignant cancer, which can easily invade adjacent areas and metastasize to lymph nodes and distant tissues (15,16). It has a high degree of prevalence and a low survival rate. Thus, it is urgent to develop molecular markers that can facilitate the early detection of ESCC.

Table VII. Correlation of *HDAC1* expression with clinicopathologic characteristics of esophageal squamous cell carcinoma in Han and Kazak patients (n=88).

Clinicopathological characteristics		Positive No. (+)			Negative No. (-)			X^2	P-value
Sex	Male	31	Han	25	31	Han	12	11.328	0.001[a]
			Kazak	6		Kazak	19		
	Female	12	Han	6	14	Han	5	0.540	0.462
			Kazak	6		Kazak	9		
Age	<60 yrs	20	Han	11	26	Han	6	4.945	0.026[a]
			Kazak	9		Kazak	20		
	≥60 yrs	23	Han	19	19	Han	12	2.036	0.154
			Kazak	4		Kazak	7		
LN Metastasis	Positive	22	Han	13	24	Han	10	1.394	0.238
			Kazak	9		Kazak	14		
	Negative	21	Han	18	21	Han	7	2.059	0.151
			Kazak	3		Kazak	14		
Differentiation	Well and moderate	36	Han	24	33	Han	11	7.654	0.006[a]
			Kazak	12		Kazak	22		
	Poor	7	Han	6	12	Han	7	1.534	0.216
			Kazak	1		Kazak	5		
Depth of invasion	T1 and T2	6	Han	3	7	Han	2	0.627	0.429
			Kazak	3		Kazak	5		
	T3 and T4	37	Han	28	38	Han	15	10.044	0.002[a]
			Kazak	9		Kazak	23		

[a]P-value was considered statistically significant at <0.05. LN, lymph node metastases; T1, tumors only invade the lamina propria and submucosa; T2, tumors invade the muscular layer; T3, tumors invade the esophageal fibrous membrane; T4, tumors invade adjacent organs; HDAC, histone deacetylase.

HDAC1 contains 482 amino acids, identified in 1996 by Taunton et al (17). HDAC1 regulates genes involved in cell differentiation and the cell cycle, and participates in the development of various diseases such as viral infectious diseases, degenerative diseases and cancer (18-20). HDAC1 can mediate site-specific DNA-binding transcriptional repression and a high level of HDAC1 is observed in tumor invasion and metastasis (21). Burdelski et al (22) identified that a high level of HDAC1 was associated with high Gleason grade, advanced pathological tumor stage, positive LN metastasis, elevated preoperative prostate specific antigen level and cell proliferation; therefore, HDAC1 expression detection may have clinical significance for the risk stratification of prostate cancer. Huang et al (23), demonstrated high expression of HDACs in cervical cancer and cervical intraepithelial neoplasia tissues. Mutze et al (24), identified high levels of HDAC1 and HDAC2 in gastric carcinomas and they were not related to the response to platinum/5-fluorouracil. High HDAC1 level is related to lower overall survival (25). There is a significant association between HDAC1 high expression and advanced age (26). High levels of HDAC1 expression are associated with a poor histological differentiation and prognosis in liver cell carcinoma (27). There is higher expression of HDAC1 in gastrointestinal malignant tumor, particularly in colorectal cancer and HDAC1 expression is closely related to clinical characteristics of gastrointestinal cancer (8). Zhong et al (28), noted that the high expression of HDAC1 may serve as a potential therapeutic target for ESCC. Miyashita et al (29), reported that the expression of HDAC1 may be involved in duodenoesophageal reflux-induced neoplastic transformation of the esophageal mucosa into cancer cells with squamous and adeno differentiation. By contrast, Langer et al (30) stated HDAC1 expression is not changed based on pT, pN category or esophageal adenocarcinoma differentiation level. Xu et al (31), noted that HERG1 contributes to poor prognosis of ESCC and suggest that targeting HERG1 may have potential diagnostic and therapeutic value for ESCC treatment. In the present study, the positive rate of HDAC1 expression in ESCC was increased compared with normal tissues. However, the expression did not change according to sex, age, metastasis, differentiation degree and invasion depth.

HDAC2 can promote tumorigenesis through several mechanisms, including promoting the degradation of β-catenin and decreasing epigenetic modification (15,32). In addition, HDAC2 can inhibit the expression of tumor suppressor p53 and p21 while promoting expression of oncogene Myc (33,34). HDAC2 participates in chronic obstructive pulmonary disease (35,36). It is also over expressed in lung

Table VIII. Correlation of HDAC2 expression with clinicopathologic characteristics of esophageal squamous cell carcinoma in Han and Kazaks patients (n=88).

Clinicopathological characteristics		Expression No. of HDAC1 mRNA						X^2	P-value
		Positive (+) No.			Negative (-)				
Sex	Male	47	Han	31	15	Han	6	3.184	0.074
			Kazak	16		Kazak	9		
	Female	21	Han	11	5	Han	0	4.540	0.033[a]
			Kazak	10		Kazak	5		
Age	<60 yrs	37	Han	17	9	Han	0	6.559	0.010[a]
			Kazak	20		Kazak	9		
	≥60 yrs	31	Han	26	11	Han	5	6.198	0.013[a]
			Kazak	5		Kazak	6		
LN Metastasis	Positive	39	Han	23	7	Han	0	8.256	0.004[a]
			Kazak	16		Kazak	7		
	Negative	29	Han	21	13	Han	4	6.461	0.011[a]
			Kazak	8		Kazak	9		
Differentiation	Well and Moderate	52	Han	32	17	Han	3	9.874	0.002[a]
			Kazak	20		Kazak	14		
	Poor	16	Han	12	3	Han	1	2.030	0.154
			Kazak	4		Kazak	2		
Depth of invasion	T1 and T2	6	Han	4	7	Han	1	3.745	0.053
			Kazak	2		Kazak	6		
	T3 and T4	62	Han	40	13	Han	3	7.544	0.006[a]
			Kazak	22		Kazak	10		

[a]$P<0.05$. LN, lymph node metastases; T1, tumors only invade the lamina propria and submucosa; T2, tumors invade the muscular layer; T3, tumors invade the esophageal fibrous membrane; T4, tumors invade adjacent organs; HDAC, histone deacetylase.

cancer (37-39). Huang et al (40), demonstrated that HDAC2 affects chromatin remodeling following DNA damage in ovarian cancer cells. It is also reported that high HDAC2 expression indicates high aggressive behavior in esophageal adenocarcinoma (30). Wang et al (41), noted that the level of HDAC2 increases dramatically in ESCC compared with adjacent non-tumor tissues. Li et al (42) reported that the HDAC2 protein level in ESCC tissues was significantly increased and was closely associated with the histological grade, invasion depth and lymph node metastasis. Göder et al (43) noted that HDAC1 and HDAC2 regulate checkpoint kinase phosphorylation through suppression of PR130. HDACi, as well as an elimination of HDAC1/HDAC2, induces PR130-dependent mechanisms that inhibit checkpoint kinase phosphorylation. However, the present study failed to detect notable changes in HDAC2 expression between ESCC and normal tissues. However, the expression of HDAC2 was related to the invasion depth.

HDAC3 is also an essential factor for cell survival and proliferation in tumors and in maintaining the structure of chromatin and the stability of genome (44). HDAC3 can form large corepressor complexes with N-CoR and SMRT (45). High HDAC3 expression has been found in colon or gastric tumor cells and it has an antiapoptotic function (46,47). High expression of HDAC3 is also noted in gastric cancer, where it is associated with poor prognosis (14). HDAC3 stimulates cell migration of ovarian carcinoma and high HDAC3 expression indicates poor prognosis of lung adenocarcinoma patients (48,49). Jiao et al (50) found that increased HDAC3 level in the nucleus, but not in the cytoplasm, was related with LN metastasis and advanced clinical staging of pancreatic cancer. It has also been reported that HDAC3 is a risk factor of ESCC and may be used to evaluate the grade of malignancy and prognosis of ESCC (51). The present study found no significant difference in the expression of HDAC3 in ESCC tissue compared with normal tissue and identified that the expression of HDAC3 was not associated with sex, age, metastasis, degree of esophageal tissue differentiation or the depth of invasion. It was hypothesized that perhaps regional differences and ethnic specificity lead to the discrepancy between the present results and a previous study (51). These results indicate that the expression of HDAC3 has no relationship with the development of ESCC.

Additionally, the present study found that the expression of HDACs in tumor tissue from Kazak people was lower compared with Han people. In ESCC, the positive rate of HDAC1 expression in ESCC tissues was increased compared with normal tissue. The expression of HDAC2 varied according to the invasion depth of ESCC patients. However, HDAC3 showed no significant expression changes in ESCC and was not correlated with the clinicopathological

Table IX. Correlation of HDA3 expressions with clinicopathologic characteristics of esophageal squamous cell carcinoma in Han and Kazak patients (n=88).

Clinicopathological characteristics				Expression No. of HDAC3 mRNA						X^2	P-value
				Positive (+) No.			Negative (-)				
Sex	Male	59		Han	37	3	Han	0		4.666	0.031[a]
				Kazak	22		Kazak	3			
	Female	23		Han	11	3	Han	0		2.487	0.115
				Kazak	12		Kazak	3			
Age	<60 years	45		Han	17	1	Han	0		0.599	0.439
				Kazak	28		Kazak	1			
	≥60 years	37		Han	28	5	Han	3		0.560	0.454
				Kazak	9		Kazak	2			
LN metastasis	Positive	43		Han	22	3	Han	1		0.357	0.550
				Kazak	21		Kazak	2			
	Negative	39		Han	25	3	Han	0		4.751	0.029[a]
				Kazak	14		Kazak	3			
Differentiation	Well and Moderate	64		Han	35	5	Han	0		5.549	0.018[a]
				Kazak	29		Kazak	5			
	Poor	18		Han	13	1	Han	0		2.287	0.130
				Kazak	5		Kazak	1			
Depth of invasion	T1 and T2	13		Han	5	0	Han	0		/	
				Kazak	8		Kazak	0			
	T3 and T4	69		Han	41	6	Han	2		1.536	0.215
				Kazak	28		Kazak	4			

[a]P<0.05. LN, lymph node metastases; T1, tumors only invade the lamina propria and submucosa; T2, tumors invade the muscular layer; T3, tumors invade the esophageal fibrous membrane; T4, tumors invade adjacent organs; HDAC, histone deacetylase.
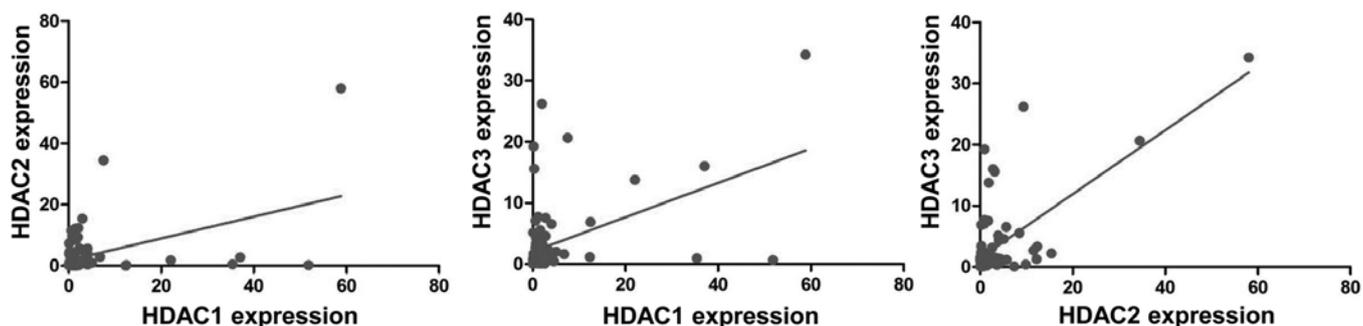

Figure 2. Correlation of *HDAC1*, *-2* and *-3* expression in human esophageal squamous cell carcinoma (n=88). Pearson's correlation test was used to analyze the correlation among *HDAC1*, *-2* and *-3*. HDAC, histone deacetylase.

characteristics of ESCC patients. The expression of HDACs in tumor tissue of Kazak people was lower compared with Han people. Additionally, it was noted that the expression of HDAC1, -2 and -3 had ethnic differences.

As for treatment, Ahrens *et al* (52) proposed that targeting epigenetic modifiers in esophageal cancers may represent a potential future therapeutic approach. Kano *et al* (53) found that CHAP31 sensitized SCC cells to carbon-ion radiotherapy and this combination treatment may be a potentially useful therapeutic strategy for ESCC. Hoshino *et al* (54) suggested that HDACi-FK228 has a potent ability to augment the effect of adenovirus-mediated p53 gene therapy in ESCC. Thus, targeting HDACs may be an effective approach for treatment of ESCC.

In summary, HDAC1 may be used as a risk factor for ESCC and HDAC2 may be used to predict ESCC invasion. The different clinical parameter expression is related to ethnic differences. Future research should focus on the effect of HDAC inhibitors on ESCC treatment in Xinjiang, China.

Acknowledgements

Funding

Availability of data and materials

All data generated or analyzed during the present study are included in this published article.

Authors' contributions

LL designed the study. HuiwL, HuiL and YW performed data collection. HuiwL and HuiL performed statistical analysis. HuiwL provided data interpretation. YinL and YikL participated in data collection of patient information. YC and LY provided help in RT-qPCR assay. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Xinjiang Medical University and informed consent was obtained from all patients.

Patient consent for publication

Not applicable.

Competing interests

All authors declare that they have no competing interests.

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