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Original Article

High expression of HDAC7 associated with MSH2 predicted a good prognosis of patients with colon cancer

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Abstract: HDAC7 belongs to class IIa HDACs, and exhibits conflicting functions in different types of cancer. Until now, the function of HDAC7 in colon cancer has not been explored. Here, we investigated the potential role of HDAC7 in colon cancer. The results indicated that HDAC7 was mainly localized in the cytoplasm with higher expression in the cancer tissues than that in the para-carcinoma tissues (10.881±4.019 VS 1.713±1.046, *P*=0.000). But the two expressions were negatively correlated, although the *P* value was borderline significant (r=-0.222, *P*=0.050). Spearman's analysis revealed that HDAC7 expression in cancer tissues was positively related to mismatch repair gene MSH2 (r=0.288, *P*=0.011), while HDAC7 expression in para-carcinoma tissues was negatively associated. Furthermore, HDAC7 expression had a significant inverse correlation with grade (r=-0.315, *P*=0.012); and MSH2 expression was negatively correlated with both N stage (r=-0.279, *P*=0.023) and cTNM stage (r=-0.241, *P*=0.033). Survival analysis revealed that colon cancer patients with high expression of HDAC7 (43.6% VS 20.0%, *P*=0.021) or MSH2 (53.3% VS 30.0%, *P*=0.015) had better prognoses in contrast with those with low expression. Taken together, these results have uncovered a previously unknown function of HDAC7 in colon cancer and might identify new diagnostic and therapeutic strategies for this disease.

Keywords: Tissue microarray, immunohistochemistry, HDAC7, MSH2, colon cancer, prognosis

Introduction

Colon cancer is the third most commonly diagnosed cancer in males and the second in females worldwide [1]. The morbidity of colon cancer has increased in recent decades in China, and the prevalence of obesity and physical inactivity caused by a westernized lifestyle may account for this trend [2]. A better understanding of the molecular mechanisms of colon cancer would support the discovery of new diagnostic and therapeutic drugs for this disease.

Homeostasis of histone and non-histone acetylation status is regulated by histone acetyl transferases (HATs) and histone deacetylases (HDACs). HDACs play a key role in modification of chromosome structure and regulation of gene expression. Acetylation of histones generally results in activation of gene transcription. Aberrant high activities of HDACs are observed in tumor cells, resulting in inhibition of gene expression including tumor suppressor genes [3].

HDAC7 belongs to class IIa HDACs, and exhibits conflicting functions in different types of cancer [4]. The few reports indicate an ambivalent role of HDAC7 in cancers: for example, HDAC7 has oncogenic functions in childhood ALL (Acute Lymphoblastic Leukemia) and pancreatic cancer [5, 6], but acts as a tumor suppressor inpro-B acute lymphoblastic leukemia (pro-B-ALL) and Burkitt lymphoma [7]. In addition, high expression of HDAC7 was related to good prognosis in lung cancer [8]. To our knowledge, the function of HDAC7 in colon cancer has not been explored.

In this present study, we investigated the potential role of HDAC7 in colon cancer by using a

Table 1. Difference of HDAC7 expression in colon cancer tissues and para-carcinoma tissues

Histology	No.	HDAC7 expression	*P-value*
Colon cancer tissue	98	10.881±4.019	0.000
Para-carcinoma tissue	80	1.713±1.046	

Mean ± Std. Deviation. The statistical differences were analyzed by NPar test, ns $P \geq 0.05$, *.

Table 2. Correlation between the expression of HDAC7 in colon cancer tissues and para-carcinoma tissues

		HDAC7 expression in para-carcinoma tissues
HDAC7 expression in colon cancer tissues	Spearman's Correlation	-0.222
	P-value	0.050
	Number	78

The relationship between the expression of HDAC7 in cancer tissues and para-carcinoma tissues was analyzed by Spearman correlation analysis.

tissue array containing 100 colon cancer tissues and 80 para-carcinoma tissues which had clinical immunohistochemical data about three mismatch repair genes (MLH1, MSH2, MSH6) and follow-up information. Immunohistochemistry and statistical analysis were used to study the association between HDAC7 expression and the occurrence, development and prognosis of colon cancer.

Materials and methods

Clinical materials

Colon cancer tissue microarray (HColA180-Su09) was obtained from Shanghai Outdo Biotech Co., Ltd., including 99 cancer tissue samples and 80 para-carcinoma tissues. The method for preparing microarrays used the standard protocol as previously published.

The colon cancer patients' age ranged from 29 to 90 with a mean age of 68, including 49 females and 50 males. The tumor size ranged from 1.5 cm to 15 cm. The surgery time was from July 2006 to May 2007 with the final follow-up in July 2015, i.e.8-9 years later. In the follow-up period, 61 patients died of colon cancer with a median follow-up time of 26 months (3-102 months); 39 patients were still alive with a median follow-up time of 102 months (98-108 months). All patients were diagnosed as having colon cancer with clinical immunohistochemistry data of mismatch repair genes (MLH1, MSH2, MSH6), and received no extra treatment before the surgery. The detailed clinical information could be found in **Table 3**.

Immunohistochemistry

Antigen retrieval was performed in citrate buffer. The tissue sections were blocked with goat serum and subsequently incubated with the primary antibody anti-HDAC7 (1:300, ab53101, Abcam) at 4°C overnight, then incubated with the secondary antibody (HRP-labeled anti-mouse antibody, DAKO), washed with PBS, visualized using diaminobenzidine (DAB) system and hematoxylin re-staining.

Randomly three positive fields of each sample were chosen and more than 3×100 cells were calculated for immunohistochemistry evaluation in the selected fields. The percentage of positive cells was scored as follows: 0% (0), 1%-20% (1), 21%-40% (2), 41%-60% (3), 61%-80% (4), 81%-100% (5). The staining intensity was scored as follows: no staining (0), week (1), moderate (2), and strong (3). The total score = staining percentage × intensity. Score ≤8 was regarded as low expression, while >8 was considered as high expression.

Statistical analysis

The difference in HDAC7 expression between colon cancer tissues and para-carcinoma tissues was analyzed by NPar test. The relationship between the expression of HDAC7 in cancer tissues and para-carcinoma tissues was analyzed by Spearman correlation analysis. The association between the expression of HDAC7 and the mismatch repair genes (MLH1, MSH2, MSH6), as well as the clinical factors were calculated by Spearman correlation analysis. The univariate analysis between HDAC7, MLH1, MSH2, MSH6, the clinical data and the survival time was estimated using the Kaplan-Meier method and log-rank test. Then, statistically significant data in univariate analysis was included in COX multivariate regression survival analysis. All statistical analyses were conducted using SPSS 17.0 software and $P<0.05$ was considered statistically significant.

Table 3. Correlation between the expression of HDAC7and the mismatch repair genes MLH1/MSH2/MSH6 in colon cancer patients

		MLH1 expression (cancer tissues)	MSH2 expression (cancer tissues)	MSH6 expression (cancer tissues)
HDAC7 expression (cancer tissues)	Spearman's Correlation	0.060	0.288	0.149
	P-value	0.597	0.011	0.183
	N	80	78	81
HDAC7 expression (para-carcinoma tissues)	Spearman's Correlation	-0.210	-0.251	-0.171
	P-value	0.073	0.034	0.145
	N	74	72	74
MLH1 expression (cancer tissues)	Spearman's Correlation	1.000	0.544**	0.481**
	P-value	.	0.000	0.000
	N	82	80	82
MSH2 expression (cancer tissues)	Spearman's Correlation	0.544**	1.000	0.713**
	P-value	0.000	.	0.000
	N	80	80	80
MSH6 expression (cancer tissues)	Spearman's Correlation	0.481**	0.713**	1.000
	P-value	0.000	0.000	
	N	82	80	83

The association between the expression of HDAC7 and the mismatch repair genes (MLH1, MSH2, MSH6) were calculated by Spearman correlation analysis. **, Correlation is significant.

Results

Expression of HDAC7 in colon cancer tissues and para-carcinoma tissues

The immunohistochemistry results indicated that HDAC7 was localized in the cytoplasm of all the samples with significantly higher expression in the colon cancer tissues than that in para-carcinoma tissues (10.881±4.019 VS 1.713±1.046, *P*=0.000) (see **Figure 1**). The detailed information is shown in **Table 1** and **Figure 1**.

Spearman's analysis revealed that the expression of HDAC7 in colon cancer tissues and adjacent tissues was negatively correlated, although the *P* value was only borderline significant (r=-0.222, *P*=0.050). Details are shown in **Table 2**.

Association between the expression of HDAC7 and the mismatch repair genes MLH1/MSH2/MSH6

The correlation between the expression of HDAC7 and the three mismatch repair genes MLH1/MSH2/MSH6 was analyzed by Spearman's correlation analysis. The results indicated that HDAC7 expression in colon cancer tissues was positively correlated with MSH2 in cancer tissues (r=0.288, *P*=0.011), while HDAC7 expression in para-carcinoma tissues showed negative correlation (r=-0.251, *P*=0.034). No significant relationship was observed between HDAC7 and MLH1 as well as MSH6 (*P*>0.050). Finally, the expressions of these three mismatch repair genes MLH1/MSH2/MSH6 were significantly positively correlated with each other (r>0, *P*=0.000). The details are shown in **Table 3**.

Correlation between HDAC7/MLH1/MSH2/MSH6 expression and clinical factors

The relationship between HDAC7/MLH1/MSH2/MSH6 expression and clinical factors of colon cancer were evaluated by Spearman's correlation analysis. The results found that HDAC7 expression in colon cancer tissues was negatively associated with histology grade (r=-0.315, *P*=0.002). Furthermore, MLH1 had significant relationship with the gender (r=0.222, *P*=0.046) and MSH2 had significantly negative correlation with lymph node stage (r=-0.256, *P*=0.023) and cTNM stage (r=-0.241, *P*=0.033), whereas MSH6 was not related to any clinical factor. The expression of HDAC7 in para-carcinoma tissues was too low to be grouped, and could not be analyzed. Detailed information is shown in **Table 4**.

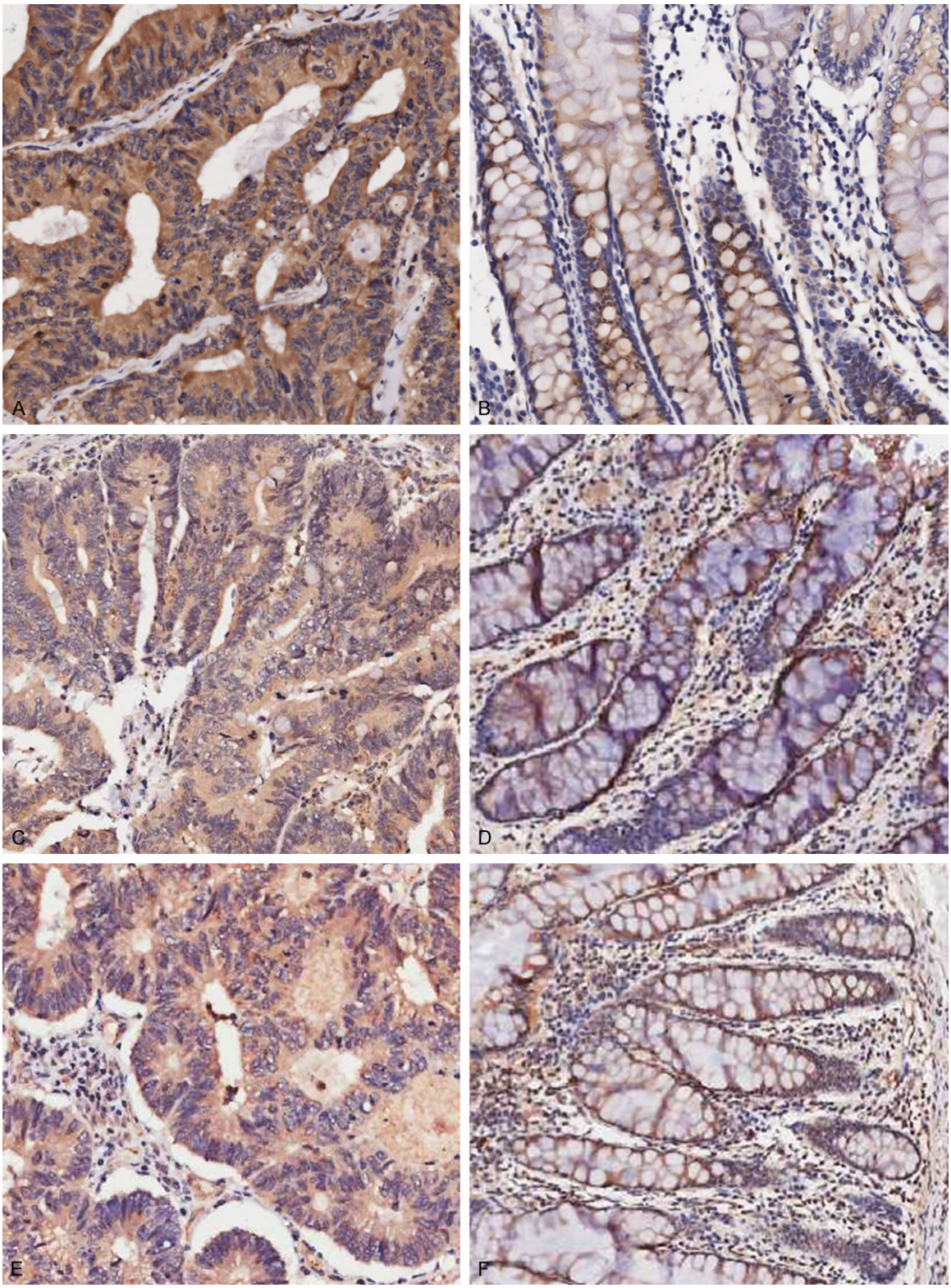

Figure 1. The immunohistochemistry indicates that HDAC7 is localized in the cytoplasm of specimen. The expression of HDAC7 in colon cancer tissues (A, C, E) was significantly higher than that in para-carcinoma tissues (B, D, F). (Magnification times: ×200).

Table 4. Correlation between clinical factors and HDAC7/MLH1/MSH2/MSH6 expression in colon cancer tissues

Clinical factors	HDAC7				MLH1				MSH2				MSH6			
	No.	expression		*P*	No.	expression		*P*	No.	expression		*P*	No.	expression		*P*
		Low	High			Low	High			Low	High			Low	High	
Gender				0.957				0.046				0.393				0.092
Male	52	9	43		44	25	19		42	26	16		42	19	23	
Female	45	10	35		37	13	24		37	23	14		37	10	27	
Lost	1	1	0		1	1	0		0	0	0		0	0	0	
Age				0.854				0.446				0.132				0.106
≤60	21	6	15		16	6	10		15	6	9		15	3	12	
>60	71	13	58		61	32	29		59	42	17		59	25	34	
Lost	6	1	5		5	1	4		5	1	4		5	1	4	
Tumor size				0.417				0.554				0.484				0.248
≤5 cm	49	8	41		41	20	21		40	24	16		40	19	21	
>5 cm	47	12	35		40	19	21		38	25	13		38	10	28	
Lost	2	0	2		1	0	1		1	0	1		1	0	1	
Grade				0.002				0.075				0.182				0.054
I	2	0	2		2	0	2		2	0	2		2	0	2	
II	46	5	41		41	17	24		41	25	16		41	13	28	
III	50	15	35		39	22	17		36	24	12		36	16	20	
T stage				0.309				0.416				0.424				0.940
T1	1	0	1		1	1	0		1	1	0		1	1	0	
T2	6	2	4		6	3	3		6	3	3		6	1	5	
T3	73	15	58		61	28	33		58	33	25		58	22	36	
T4	14	2	12		12	5	7		12	10	2		12	4	8	
Lost	4	1	3		2	2	0		2	2	0		2	1	1	
N stage				0.106				0.396				0.023				0.743
N0	58	10	48		51	22	29		49	25	24		49	17	32	
N1	27	4	23		21	13	8		21	18	3		21	9	12	
N2	11	5	6		9	3	6		8	6	2		8	3	5	
Lost	2	1	1		1	1	0		1	0	1		1	0	1	
M stage				0.392				0.160				0.245				0.114
M0	95	18	77		80	37	43		78	48	30		78	28	50	
M1	3	2	1		2	2	0		1	1	0		1	1	0	
cTNM				0.202				0.545				0.033				0.512
1	6	1	5		6	4	2		6	4	2		6	2	4	
2	52	9	43		45	18	27		43	21	22		43	15	28	
3	35	7	28		28	14	14		28	23	5		28	11	17	
4	3	2	1		2	2	0		1	1	0		1	1	0	
Lost	2	1	1		1	1	0		1	0	1		1	0	1	

The clinical factors were calculated by Spearman correlation analysis, and the univariate analysis was estimated using the Kaplan-Meier method.

High expression of HDAC7 and MSH2 correlated with improved prognosis of colon cancer patients

Kaplan-Meier analysis and log-rank test were used to uncover the association between HDAC7 expression, MLH1/MSH2/MSH6 expression, clinical factors and the overall survival time of colon cancer patients. The results revealed that HDAC7 expression in cancer tissues was significantly positively associated with the overall survival time. That indicated

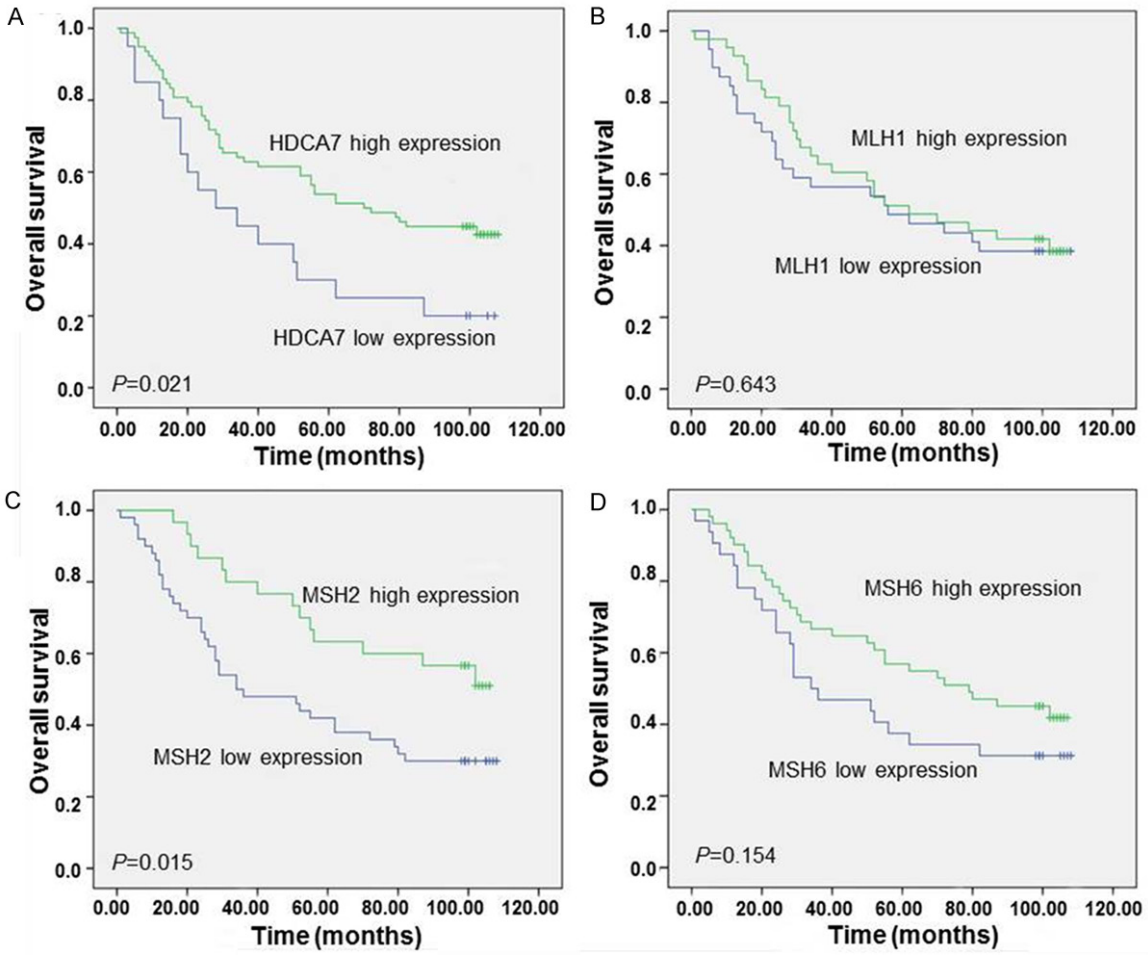

Figure 2. Kaplan-Meier survival curves dependent on (A) HDAC7 expression, (B) MLH1 expression, (C) MSH2 expression, (D) MSH6 expression. *P* values were calculated with log-rank test.

that the colon cancer patients with high HDAC7 expression had a longer survival time than those with low HDAC7 expression. A similar relationship was also observed between MSH2 expression and prognosis, although no correlations were found between MLH1, MSH6 and the prognosis. Meanwhile, N stage, M stage and cTNM were all negatively correlated with the patients' prognosis, while gender, age, tumor size, grade and T stage did not correlate. The details are shown in **Figure 2**.

Subsequently, we included these five prognostic indices into COX multivariate regression survival analysis. The result indicated no single independent prognostic factor ($P>0.050$). Detailed analysis is shown in **Table 5**.

Discussion

In this paper, we report an investigation of the association between HDAC7 expression and colon cancer using immunohistochemistry test and statistical analysis.

The results indicated that HDAC7 was mainly localized in the cytoplasm with significant higher expression in the cancer tissues than that in the para-carcinoma tissues, which is in consistent with previous study [5, 6]. We also found evidence suggesting an inverse relationship between HDAC7 expression in cancer tissues and para-carcinoma tissues. Subsequent analysis indicated that HDAC7 expression in cancer tissues was positively related to MSH2, while HDAC7 expression in para-carcinoma tissues showed negative correlation. Based on these results, we propose that HDAC7 is involved in different gene regulatory networks in cancer tissues and para-carcinoma tissues, and may be affected by MSH2 with positive regulation and negative regulation respectively. To validate this hypothesis, we further analyzed the

Table 5. Cox regression analysis under inclusion of clinical factors and HDAC7/MSH2 expression

	Overall survival	
	HR (95% CI)	*P*
HDAC7 expression	0.635 (0.299-1.351)	0.238
MSH2 expression	0.535 (0.267-1.071)	0.078
N	1.327 (0.623-2.826)	0.464
M	2.982 (0.510-17.447)	0.225
cTNM	1.397 (0.585-3.335)	0.452

COX multivariate regression survival analysis was estimated using the Kaplan-Meier method and log-rank test.

correlation between HDAC7/MLH1/MSH2/MSH6 expression and the clinical factors of the patients. The results showed that HDAC7 expression had a significant inverse correlation with histology differentiation; meanwhile MSH2 expression was also negatively correlated with both N stage and cTNM stage. Survival analysis revealed that the colon cancer patients with high expression of HDAC7 or MSH2 had better prognoses in contrast with those with low expression. Moreover, N, M and cTNM stage were all significantly associated with prognosis. Unfortunately, none of these potential prognostic markers was an independent predictive factor, which might relate to their significant interrelationships. However, these results supported the above hypothesis.

Although most previous reports predicted the potential carcinogenicity function of HDAC7, such as inhibition of osteodastogenesis which may result in cancer bone metastasis, promotion of angiogenesis by inhibiting angiogenesis suppressor gene AKAP12 [9, 10], there were still a few reports about the anti-oncogenic function of HDAC7 in some types of cancers. For instance, Barneda and colleagues demonstrated that HDAC7 induced tumor cells apoptosis by interacting with MEF2C, an oncogene that inhibited tumor cell apoptosis. HDAC7 was also demonstrated to downregulate c-Myc transcription in SD-1 and Namalwa cells; this is a known oncogene encoding a transcription factor and having a critical role in the proliferation of cancer cells [7]. Unlike these previous reports, our experiments firstly confirmed a positive association in colon cancer between HDAC7 and MSH2, an important member of the mismatch repair gene family. Mismatch repair is a mechanism involved in DNA damage replication by correcting DNA biosynthetic errors, thus functioning as tumor suppression. Mismatch repair genes (MLH1/MSH2/MSH6) are crucial to DNA mismatch repair function, and show strong association with colon cancer development, including drug resistance and heredity which are important for specific therapy of colon cancer [11, 12]. We hypothesized that HDAC7 might participate in a DNA mismatch repair process through interacting with MSH2 in colon cancer, thus reducing the malignant degree and lymph node metastasis ability of tumor cells, and improving the survival time of patients.

In conclusion, our findings first demonstrated the tumor suppressive function of HDAC7 in colon cancer and revealed a significant link between HDAC7 and DNA repair gene MSH2. Of note, HDACs are commonly recognized as carcinogenic, due to the carcinogenicity of most HDACs function. HDAC inhibitors have gained much attention as tumor therapeutic agents, due to their function of recruiting the acetylation of histone and non-histone proteins and thus promoting tumor cells apoptosis [13]. However, our finding in this paper predicts that HDAC7 is a tumor suppressor in colon cancer. Thus, HDAC7 inhibitors may not be used as therapeutic agents for all types of cancers due to the complex functions of HDAC7 with tumor- and tissue-specificity. In the future we plan to construct colon cancer cell lines and investigate the regulatory network of HDAC7 in colon cancer by gene knock-down or over-expression.

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Disclosure of conflict of interest

None.

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