Original Article
Expression of CHD5 may serve as an independent biomarker of prognosis in colorectal cancer via immunohistochemical staining

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Abstract: Chromodomain helicase DNA binding protein 5 (CHD5) acts as a tumor suppressor in various types of cancer and belongs to CHD protein family. However, no prognostic role for CHD5 has yet been indicated in colorectal cancer. Therefore, the aim of this study was to investigate a possible association between CHD5 expression and colorectal cancer prognosis. Furthermore, immunochemistry was used to investigate CHD5 expression in 310 CRC tissue specimens. Expression of CHD5 significantly positively correlated with the lymphatic metastasis (P=0.007). The prognostic value of CHD5 in relation to overall survival was analyzed by Kaplan-Meier analysis and Cox proportional hazard models. The mean and medium follow-up times after surgery were 5.5 and 6.6 years, respectively. A total of 150 patients died during the 13 years of follow-up in the survey period. We also demonstrated that overall survival was poor in CRC patients with low expression of CHD5 (P=0.003). Accordingly, multivariate analysis identified low CHD5 expression as an independent risk factor (P=0.014), especially in elderly patients or those with late stage cancers. We suggest that CHD5 could serve as an independent prognostic biomarker for colorectal patients. This finding also should be verified by other research groups.

Keywords: CHD5, prognosis, colorectal cancer, immunohistochemistry staining

Introduction

Colorectal cancer (CRC) ranks third in human cancer deaths, which is also the fourth most common cancer cause of death globally, accounting for roughly 1.2 million new cases and 600000 deaths per year [1]. To the best of knowledge, cancer patient survival is largely dependent on early diagnosis and intervention [2]. Moreover, the Stage at Diagnosis in the CRC patients is the most important prognostic factor [3]. Additionally, tumor metastasis and recurrence also seriously influence patients' prognosis and quality of life. At present, however, most CRC patients are often diagnosed at a late stage, which includes lymph node invasion or distant metastasis, resulting in poor prognosis.

Therefore, there is an urgent need for the identification of prognostic biomarkers that can identify CRC at earlier stages or predict the recurrence and metastasis of CRC. Recent studies showed that CHD5 was expressed in all types of adenoma either epigenetically or by chromosomal deletion and likely acted as a tumor suppressor gene in early colorectal carcinogenesis [4]. In the present study, we therefore explored the potential availability of CHD5 in the prognosis of CRC patients.

The CHD family of proteins consisted of nine members: CHD1-CHD9, which make up two N-terminal chromodomains, a helicase-like ATPase motif associated with nucleosome remodeling, and a less well-defined C-terminal DNA binding domain [5]. Among these members, CHD5 gene located on 1p36 encodes a protein-chromodomain helicase DNA-binding protein 5 [6] and firstly identified in the nervous system, which played a role in the pathogenesis of neural tumors [7]. Subsequently, Garcia et al.
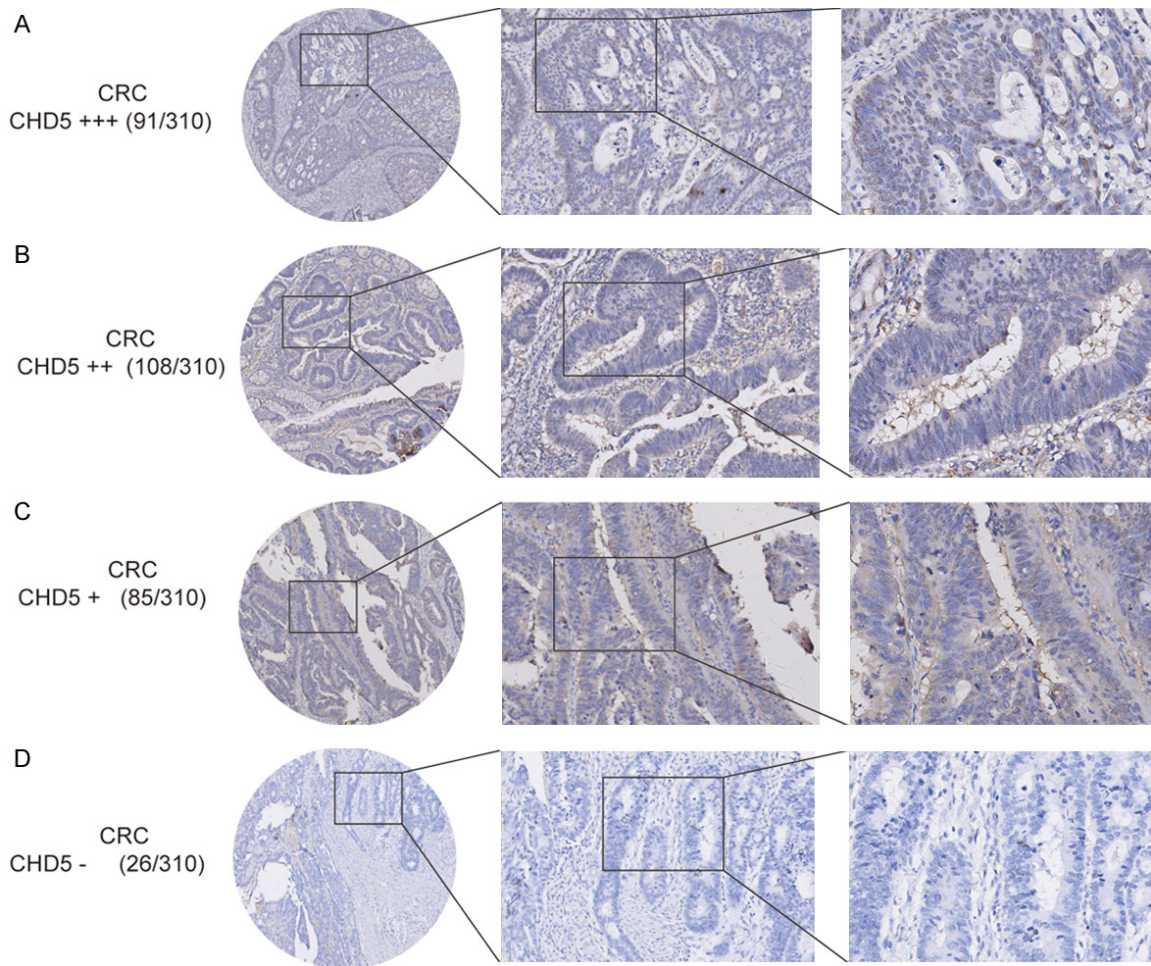
Figure 1. Representative immunostaining of CHD5 in CRC tissue samples. A: CRC, scored as (+++); B: CRC, scored as (++); C: CRC, scored as (+); D: CRC, scored as (-). Representative images are shown at 200× and 400× magnification, respectively.

showed that CHD5 was an independent marker of outcome in neuroblastoma, as a tumor suppressor gene [8]. Later research gradually confirmed that CHD5 also functioned as a tumor suppressor gene in a variety of other tumor types, such as breast, colon, lung, ovary and prostate cancers [9]. Ma et al. drew a conclusion that downregulation of CHD5, which was mediated by abnormal methylation, contributed to the development and progression of breast cancer [10]. Similarly, CHD5 may also act as a tumor suppressor gene in non-small cell lung cancer (NSCLC) [11]. In addition, it was found that CHD5 was downregulated in a certain number of ovarian cancers and appeared to be an adverse predictor candidate of ovarian cancer disease-free and total survival [12]. Although downregulating CHD5 expression played a role in colorectal tumorigenesis [13], its prognostic role remains controversial.

In this study, we therefore explored the possibility of a potential prognostic role for CHD5 in colorectal cancer.

Materials and methods

Clinicial tissue samples

All samples were obtained following the participants' written informed consent, and all experiments were approved by the local Ethics Committee of the Shanghai Jiao-Tong University School of Medicine at Renji Hospital. Tissue microarrays consisting of 310 CRC specimens with confirmed histological diagnosis were obtained from Renji Hospital (Shanghai, China) from January 2003 to December 2012. The follow-up duration was calculated from the date of surgery to the date of death or the last known follow-up. None of these patients had received radiotherapy, chemotherapy, or any other relat-

Table 1. Relationship of CHD5 expression with clinical parameters in 310 colorectal cancer patients

Variable		CHD5 (n)		P[1]
		Low	High	
		n=111 (%)	n=199 (%)	
Age (years)	≤65	56 (50.45)	102 (51.26)	0.892
	>65	55 (49.55)	97 (48.74)	
Gender	Male	60 (54.05)	118 (59.30)	0.371
	Female	51 (45.95)	81 (40.70)	
Tumor size	≤5 cm	65 (58.56)	123 (61.81)	0.183
	>5 cm	51 (41.44)	76 (38.19)	
Tumor location	Rectum	100 (90.09)	189 (94.97)	0.101
	Colon	11 (9.91)	10 (5.03)	
Serum CEA	≤5 ng/ml	55 (49.55)	102 (51.26)	0.773
	>5 ng/ml	56 (50.45)	97 (48.74)	
Tumor infiltration	T1	6 (5.41)	5 (2.51)	0.318
	T2	20 (18.02)	33 (16.58)	
	T3	31 (27.92)	46 (23.12)	
	T4	54 (48.65)	115 (57.79)	
Lymphatic metastasis	N0	62 (55.86)	115 (57.79)	**0.007**
	N1	23 (20.72)	62 (31.16)	
	N2	26 (23.42)	22 (11.05)	
Distant metastasis	M0	95 (85.59)	179 (89.95)	0.250
	M1	16 (14.41)	20 (10.05)	
Clinical stage	I	18 (16.22)	27 (13.57)	0.451
	II	46 (41.44)	86 (43.21)	
	III	31 (27.93)	67 (33.67)	
	IV	16 (14.41)	19 (9.55)	

Values in parentheses indicate percentage values. The bold number represents the *P*-values with significant differences. [1]*P* Value was calculated by χ^2 test.

follows: "-" for a score of 0-2, "+" for a score of 3-5, "++" for a score of 6-8 and "+++" for a score of 9-12; and low expression was defined as a total score <6 and high expression with a total score ≥6. These scores were determined independently by two senior pathologists. The scoring by the pathologists was done in a blinded manner.

Statistical analysis

Statistical analyses were performed using SPSS version 24.0 (SPSS Inc, Chicago, USA) and GraphPad Prism 6 (San Diego, CA) software. The Chi-square test was used to analyze clinicopathological characteristics. Survival curves were evaluated using the Kaplan-Meier method, and analyzed by the log-rank test. Univariate and multivariate Cox regression analysis were performed to identify the prognostic factors. All the experiments were repeated at least three times. *P* values <0.05 were considered to be statistically significant.

Results

CHD5 is expressed in the majority of colorectal cancer specimens and locates to the nucleus

ed anti-tumor therapy before surgery. Clinical data were obtained from previous data base, including gender, age, stage, T, N, and M stages, and follow-up information.

Immunohistochemical staining

Immunohistochemical staining was performed as described. The CHD5 antibody was purchased from Abcam (1:50, Cambridge, UK). Protein expression was quantified using a visual grading system based on the extent and intensity of staining. The percentage of positive tumor cells was graded on the following 0-4 scale: 0, none; 1, 1-25%; 2, 26-50%; 3, 51-75%; 4, 76-100%. The staining intensity was graded on the following 0-3 scale: 0, no staining; 1, weak staining; 2, moderate staining; 3, strong staining. The final score was designated as low or high expression group using the percent of positive cell score × staining intensity score as

We validated a role for CHD5 in CRC patients by evaluating its expression from 310 patients. CHD5 expression was evaluated by Immunohistochemical (IHC) staining of tissue arrays. **Figure 1A-D** showed a representative staining for CHD5 in colorectal tumor specimens. We found that CHD5 was expressed in the majority of colorectal cancer specimens and located to the nucleus. Furthermore, CHD5 was highly expressed in the 199 (64.19%) of the CRC samples, whereas the remaining 111 (35.81%) samples had a low expression level (**Figure 1**).

CHD5 expression is associated with clinicopathological features

To evaluate the clinical significance of CHD5, the chi-square test was used to analyze the associations with CHD5 expression and clini-
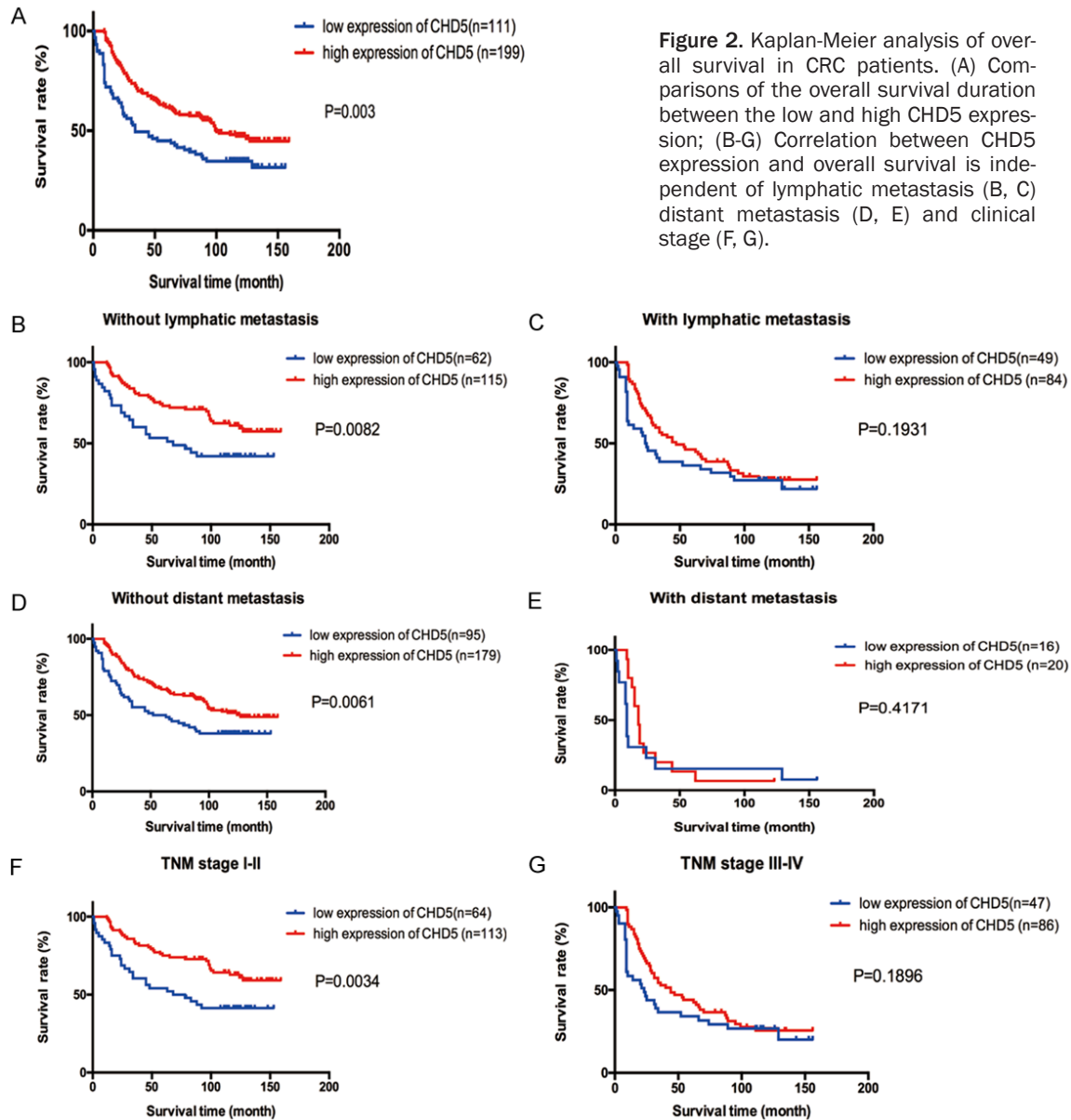
Figure 2. Kaplan-Meier analysis of overall survival in CRC patients. (A) Comparisons of the overall survival duration between the low and high CHD5 expression; (B-G) Correlation between CHD5 expression and overall survival is independent of lymphatic metastasis (B, C) distant metastasis (D, E) and clinical stage (F, G).

copathological characteristics of CRC patients. CHD5 expression in CRC tissues was closely correlated with lymph mode metastasis (P= 0.007). However, no significant correlation was detected between CHD5 expression and age, gender, tumor size, tumor location, serum CEA levels, tumor infiltration, distant metastasis, and clinical stage (**Table 1**). This staining pattern indicated that CHD5 expression was possibly correlated with carcinogenesis and invasion of colorectal cancer.

The prognostic role of CHD5 expression in colorectal cancer patients

We determined a prognostic role for CHD5 expression in colorectal cancer by collecting overall survival data from 310 patients. The mean and median follow-up times after surgery were 5.5 and 6.6 years (ranging from 0.1 to 13.2 years), respectively. During the 13 years of follow-up in this survey, 150 (48.4%) patients died and the five-year survival rate was 55.8%. To determine the prognostic value of CHD5 for CRC, we used the Kaplan-Meier survival curves and the log- rank test to evaluate the relationship between CHD5 expression and the clinical follow-up data. The results revealed that low expression of CHD5 is negatively associated with overall survival (OS) (P < 0.001, **Figure 2A**), which indicated that the OS was poor in CRC patients with low CHD5 expression. We also determined that low expression of CHD5 was

Table 2. Univariate and multivariate analyses of prognostic parameters for survival in 310 colorectal cancer patients

Variable	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P* value	HR	95% CI	*P* value
CHD5	0.598	0.429-0.835	**0.003**	0.646	0.456-0.916	**0.014**
Age	1.591	1.139-2.222	**0.006**	1.897	1.340-2.687	**<0.001**
Gender	1.242	0.894-1.726	0.196	-	-	-
Size	1.231	0.883-1.716	0.221			
Location	1.598	0.813-3.140	0.174	-	-	-
CEA	1.114	0.802-1.547	0.521	-	-	-
T	1.354	1.095-1.675	**0.005**	1.223	0.950-1.575	0.119
N	1.689	1.381-2.065	**<0.001**	1.286	0.912-1.813	0.151
M	4.105	2.657-6.342	**<0.001**	2.383	1.236-4.593	**0.010**
TNM	1.913	1.561-2.343	**<0.001**	1.166	0.759-1.792	0.484

HR: Hazard ratio; CI: Confidence interval. The bold number represents the *P*-values with significant differences.

related with poorer overall survival, in terms of lymph node metastasis, distant metastasis and clinical stage (**Figure 2B-G**).

Prognostic role of CHD5 expression according to the clinicopathological characteristics of colorectal cancer

To directly identify the risk factors associated with OS in CRC patients, univariate and multivariate analysis were performed to confirm that CHD5 represented an independent risk factor for poor prognosis. Univariate Cox regression analysis showed that CHD5 expression level, age, tumor classification, lymphatic metastasis, and distant metastasis were significantly associated with OS (**Table 2**). Furthermore, multivariate Cox regression analysis confirmed that CHD5 expression level, age and distant metastasis were independent predictors of OS in patients with CRC (**Table 2**). These data indicate that low expression of CHD5 may be a predictor for diagnosis and prognosis in colorectal cancer patients.

Discussion

Currently, nine members of the CHD family have been shown to promote cancer progression [14]. In regarding the gastroenteric tumor, Kim et al. suggested that frameshift mutation and loss of expression of CHD genes are common in gastric cancer and colorectal cancer with MSI-H, which might contribute to cancer pathogenesis by deregulating CHD-mediated chromatin remodeling [15]. CHD4 may play an

important role in monitoring the clinical behavior of colorectal cancer, as a biomarker of prognosis [16] and CHD8 had been demonstrated as a novel indicator for biological aggressiveness in gastric cancer [17]. Similarly, CHD5 had been found as a tumor suppressor gene in early CRC. Interestingly, CHD5 also acted as an independent prognostic factor in the epithelial ovarian cancer and neuroblastoma, respectively [12, 18]. However, a prognostic role has not been previously demonstrated for CHD5 in colorectal cancer. Therefore, it is reasonable to hypothesize that CHD5 may serve as a prognostic biomarker in CRC patients.

To the best of our knowledge, this is the first study to report a potential prognostic role for CHD5 in colorectal cancer. More recently, we found downregulation of CHD5 at the RNA level in colorectal cancer cells, which suggested that CHD5 might reduce the migration and invasion [4]. Then, in the current study, we report that CHD5 expression was detected mainly in colorectal tumor tissue, which located at the nucleus by IHC staining (**Figure 1A-D**). Furthermore, the expression of CHD5 in CRC samples was positively correlated with lymphatic metastasis (**Table 1**). Importantly, Kaplan-Meier survival analysis showed patients displaying a low CHD5 expression level exhibited significantly shorter survival duration than those displaying a high CHD5 expression level, which proved that CHD5 may function as a tumor suppressor gene in colorectal cancer (**Figure 2A-G**).

Moreover, we determined that patients with low CHD5 expression in the tumor portion had significantly poor prognosis. Further analysis showed that CHD5 had a more significant association with clinical outcome in elderly patients and in patients with late stage cancers (**Table 2**). This indicated that the prognostic role of CHD5 might differ among patients with specific clinicopathological characteristics (**Table 2**).

In conclusion, our study elucidates that CHD5 expression could serve as an independent

prognostic factor in CRC patients, in terms of overall survival, as well as an important clinical biomarker for the guidance in colorectal cancer diagnosis and treatment. However, we could not determine exact mechanism that why patients with low CHD5 expression would have unfavorable clinical outcomes. Further research of the possible mechanism of CHD5 in colorectal cancer is warranted.

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Disclosure of conflict of interest

None.

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