# Original Article CD44v6 expression in patients with stage II or stage III sporadic colorectal cancer is superior to CD44 expression for predicting progression

LH Zhao, QL Lin, J Wei, YL Huai, KJ Wang, HY Yan

Department of Pathology, Beijing Civil Aviation Hospital, 1 Gao Jing Jia, Chao Yang District, Beijing 100123, China

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**Abstract:** Background: Currently, it is difficult to predict the prognosis of patients exhibiting stage II or stage III colorectal cancer (CRC) and to identify those patients most likely to benefit from aggressive treatment. The current study was performed to examine the clinicopathological significance of CD44 and CD44v6 protein expression in these patients. Study design: We retrospectively investigated 187 consecutive patients who underwent surgery with curative intent for stage II to III CRC from 2007 to 2013 in the Beijing Civil Aviation Hospital. CD44 and CD44v6 protein expression levels were determined using immunohistochemistry and compared to the clinicopathological data. Results: Using immunohistochemical detection, CD44 expression was observed in 108 (57.75%) of the CRC patients; and its detection was significantly associated with greater invasion depth, lymph node metastasis, angiolymphatic invasion, and a more advanced pathological tumor-lymph node-metastasis (TNM) stage. CD44v6 expression was observed in 135 (72.19%) of the CRC patients; and its expression was significantly associated with a poorly differentiated histology, greater invasion depth, lymph node metastasis, angiolymphatic invasion, greater invasion depth, lymph node metastasis, angiolymphatic invasion, and a more advanced pathological tumor-lymph node metastasis, angiolymphatic invasion, and a more advanced pathological tumor stage and its expression was observed in 135 (72.19%) of the CRC patients; and its expression was significantly associated with a poorly differentiated histology, greater invasion depth, lymph node metastasis, angiolymphatic invasion, and a more advanced pathological TNM stage. Expression of CD44v6 was higher than that of CD44 in stage II and stage III sporadic CRC. Conclusion: CD44v6 is a more useful marker for predicting a poor prognosis in stage II and stage III sporadic CRC as compared to CD44.

Keywords: CD44, CD44V6, colorectal cancer, immunohistochemistry

#### Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide and the leading cause of cancer mortality in western countries [1]. During the past several decades, the incidence of CRC has changed remarkably in China, as this country has experienced a two to four times increase in the incidence of CRC. From 2000 to 2005, the total number of CRC cases has increased by 19.1% and 17.7% in Chinese males and females, respectively [2]. Furthermore, in China, the incidence is still growing. and it is now the fifth leading cause of cancer mortality [3]. The size of the Chinese population makes it imperative to investigate the usefulness of specific histopathological markers for CRC progression, specifically in this population.

CD44 comprises a group of single gene-derived transmembrane glycoprotein molecules that

are widely expressed in normal epithelial, mesenchymal, and hematopoietic cells and function as cell surface hyaluronan receptors. Constituting one of the five major families of cell adhesion molecules, CD44 has attracted much interest due to its role in cancer and stem cell biology [4]. It is encoded by a single gene containing 20 exons and is located on the short arm of chromosome 11 (11p13) [5]. Alternative splicing and differential glycosylation generate many different isoforms of CD44 with often highly specific expression patterns. The interaction between CD44 molecules and hyaluronan mediates both cell-cell and cell-extracellular matrix interactions. All isoforms contain a constant region comprising a large ectodomain (270 amino acids), a transmembrane domain (23 amino acids), and a cytoplasmic domain (72 amino acids). These regions are encoded by the first five exons and the last exons (16-20), accounting for the smallest but ubiquitously expressed isoform CD44. Close to the trans-

Stage	T Stage	N Stage	M Stage
I	T1 or T2	NO	MO
lla	ТЗ	NO	MO
llb	Τ4	NO	MO
Illa	T1 or T2	N1	MO
IIIb	T3 or T4	N1	MO
IIIc	Any T	N2	MO
IIId	Any T	N3	MO
IIIc	Any T	N4	MO
IV	Any T	Any N	M1

 Table 1. Tumor classification

membrane region, a variable part encoded by various combinations of exons 6-15 (v1-v10) can be included, giving rise to CD44 variant isoforms (CD44v) [6]. Engagement of CD44 with its cognate ligand is known to induce tumor necrosis factor (TNF) and interleukin-1 (IL-1) production and release [8]. Thus, the CD44 family is important in a variety of physiological and pathological processes, including wound healing and leukocyte extravasation at sites of inflammation. In cancer biology, the molecule has attracted interest because of its presumed role in the development of metastasis [7]. In apparent agreement, numerous studies have demonstrated the association between CD44 expression and poor patient survival in multiple cancer types, including colon cancers [9].

The variant isoform CD44v6 has evoked special interest. Transfection of splice variants CD44v4-v7 confers metastatic potential to cells of a nonmetastatic rat tumor cell line [10]. CD44v6, like all other isoforms, contains a hyaluronan-binding site in its extracellular domain and thereby serves as a major cell surface receptor for hyaluronan [11]. CD44v6 plays an important role in tumor invasion and metastasis by regulating the extracellular matrix, promoting cell motility, and suppressing tumor apoptosis [12]. Furthermore, CD44v6 is thought to be positively correlated with invasive growth and metastasis development in some tumors [13]. Saito et al. demonstrated a correlation between CD44v6 expression and both tumor progression and poor prognosis in CRC [14]. However, this observation remains largely unconfirmed and, in particular, its significance in the Chinese population remains unknown. In the present study, we examined the expression levels of CD44 and CD44v6 protein in stage II and stage III sporadic CRC by immunohistochemistry and analyzed the clinicopathological significance.

## Materials and methods

#### Case selection and tissue samples

A total of 187 patients with primary sporadic colorectal adenocarcinoma who underwent resection with curative intent at the Beijing Civil Aviation Hospital between January 2007 and December 2013 were selected from a prospectively collected database on the basis of the availability of resected tissue. The protocol was approved by the appropriate Institutional Review Board, and informed consent was obtained from all patients in accordance with institutional regulations. All 187 patients were diagnosed as having stage II or stage III sporadic colorectal adenocarcinoma. No patient had received any prior therapy, such as radioor chemotherapy. All hematoxylin-eosin-stained sections were reviewed, the quality of the material was checked, and the best section from each specimen was selected. All cases were histologically confirmed as CRC and reviewed by two pathologists. Cancer-specific data evaluated for each patient included stage at presentation, tumor grade, specific histology, tumor location, number of tumor-positive lymph nodes, and presence of metastases. Each tumor stage was coded as described by the American Joint Commission on Cancer (AJCC), sixth edition, according to the tumor-lymph node-metastasis (TNM) staging system (T1, tumor invades submucosa; T2, tumor invades muscularis propria; T3, tumor invades through the muscularis propria into the subserosa or into nonperitonealized pericolic tissues; T4, tumor invades other organs or structures directly and/or perforates visceral peritoneum; NO, no regional lymph node metastasis; N1, metastasis to one to three regional lymph nodes; N2, metastasis to four or more regional lymph nodes; M0, no distant metastasis; M1, distant metastasis).

# Staging methodology

The tumors were classified according to **Table 1**. Clinical data including sex and age were obtained by chart review. Clinicopathological characteristics of these sporadic CRC cases were collected and are presented in **Table 2**. All cases included in this study were neoadjuvant therapy naive.

	NI		CD44		P value	CD44v6		X <sup>2</sup>	P value
	IN	-	+ (%)			-	+ (%)		
Colorectal cancer	187	79	108 (57.75)	35.546	0.000	52	135 (72.19)	82.883	0.000
Pericancerous tissue	187	136	51 (27.3)			140	47 (25.1%)		

Table 2. Comparison of CD44 and CD44v6 expression in colorectal cancer and pericancerous tissue

 
 Table 3. Comparison of CD44 and CD44v6 expression in stage II and stage III sporadic colorectal cancer

	Ν	Stage II		2	Dualua		Stage III		Dualua
		-	+ (%)	X-	Pvalue	-	+ (%)	X-	r value
CD44	187	54	70 (56.45)	4.424	0.035	25	38 (60.32)	4.493	0.034
CD44v6	187	38	86 (69.35)			14	49 (77.78)		

#### Immunohistochemistry

Tissue samples were fixed in 10% formalin, dehydrated in ethanol, and embedded in paraffin wax. For routine pathological examination, all specimens were sliced continuously into 4-µm-thick sections, stained with hematoxylin and eosin, and examined independently by two pathologists.

Immunohistochemical staining was performed using the streptavidin-peroxidase method according to the manufacturer's instructions (MaiXin Biotechnology Company, Fuzhou, China). After the sections had been deparaffinized and rehydrated in a descending graded series of alcohol dilutions, they were heated in an 800-W microwave oven at maximum power for 5 min in 0.01 M citrate buffer (pH 6.0) for antigen retrieval and then cooled to room temperature. After blocking with goat serum (Mai-Xin Biotechnology Company, FuZhou, China), the sections were incubated with primary mouse anti-human antibodies to CD44 (MaiXin Biotechnology Company, FuZhou, China) or CD44v6 (MaiXin Biotechnology Company, FuZhou, China) for 20 min at room temperature. Next, the slides were washed three times using 0.1 M phosphate-buffered saline (PBS; pH 7.4) and incubated with a biotinylated horseradish peroxidase goat anti-mouse secondary antibody (MaiXin Biotechnology Company, FuZhou, China) for 20 min at 37°C. Slides were washed three times after incubation with the secondary antibody using 0.1 M PBS (pH 7.4). Immunolabeling was visualized with 0.05% diaminobenzidine (MaiXin Biotechnology Company, FuZhou, China) in 0.01 M PBS (pH 7.4) for 5 min at room temperature, and the slides were then rinsed for 2 min under running tap water. The immunohistochemical staining was examined and photographed (at 100× and 400× magnification) using a Nikon 80i light apan). The percentage of

microscope (Nikon, Japan). The percentage of positive cells was established semiquantitatively by counting the number of labeled cells in 10 randomly selected high power fields for each specimen, at 400× magnification. As expected, CD44 and CD44v6 immunostaining was localized in the cytoplasm and plasma membrane. As a positive control for both CD44v6 and CD44 staining, breast cancer specimens were used, which were provided by the Department of Pathology, Civil Aviation Hospital, to act as positive control samples. As a negative control, slides in which the primary antibody was replaced with 0.1 M PBS (pH 7.4) were used.

## Scoring of the slides

CD44 and CD44v6 immunohistochemical staining was assessed independently by two observers, blinded to the clinical data. For each specimen, a composite immunohistochemical score (IHC) was established for both CD44 and CD44v6. This score took into account the percentage of stained cells (0-100%) as well as the staining intensity. The scoring system used for both CD44 and CD44v6 expression was similar to previously published methods [5]. The size of the positively stained compartment was estimated and classified on a five-point positive range score as follows: grade 0, 0-5%; grade 1, 6-25%; grade 2, 26-50%; grade 3, 51-75%; grade 4, > 75%. Positive extent score: 0, no staining; 1, light yellow; 2, brown; 3, dark brown. The positive range score and positive extent score were subsequently added and thus yielded a composite quantitative score: < 2, negative (-); 2-3, slight positive (+); 4-5, moderately positive (++); and 6-7, strongly positive (+++).

		CD44		2	Р	CD44v6		2	Р
	n	-	+	X-	value	-	+	X-	value
Sex									
Male	116	47	69	0.374	0.541	32	84	0.007	0.931
Female	71	32	39			20	51		
Age (years old)									
≤ 40	5	2	3	1.394	0.845	1	4	1.224	0.874
41-50	16	6	10			4	12		
51-60	62	25	37			16	46		
61-70	73	30	43			20	53		
> 70	31	16	15			11	20		
Туре									
Ulcerated type	156	64	92	0.574	0.449	44	112	0.074	0.785
Protruded type	31	15	16			8	23		
Tumor size (cm)									
< 3	31	15	16	0.657	0.720	10	21	3.273	0.195
3-5	102	41	61			32	70		
> 5	54	23	31			10	44		
Location									
Right colon	47	20	27	0.062	0.969	15	32	2.705	0.259
Left colon	61	25	36			20	41		
Rectum	79	34	45			17	62		
Degree of differentiation									
High	20	10	10	2.315	0.314	9	11	6.688	0.035
Moderate	109	41	68			34	75		
Low	58	28	30			10	48		
Invasion depth									
Intramuscularis	39	20	19	7.194	0.028	15	24	8.830	0.012
Intrasubserosa	88	42	46			36	52		
Extrasubserosa	60	17	43			11	49		
Angiolymphatic invasion									
Without	123	61	62	7.952	0.005	41	82	5.467	0.019
With	64	18	46			11	53		
Lymph node metastasis									
0	124	61	63	7.658	0.022	42	82	7.220	0.027
1-3	49	13	36			8	41		
≥ 4	14	5	9			2	12		

**Table 4.** Correlation between CD44 and CD44v6 expression with respect to clinicopathology

For statistics, all the samples that expressed CD44and CD44v6 form (+) to (+++) were regarded as positive.

#### Pathological examination

Standard pathological analysis was performed by two gastrointestinal pathologists who were blinded to the clinical data. After the final histological examination, the tumor was staged two biomarkers were estimated. Significance was at the level of P < 0.05.

according to the six-

th edition of the AJ-

CC TNM staging system. Resection specimens were evaluated for depth of tumor penetration, lymph node involve-

type, lymphatic invasion, vascular invasion, and perineural invasion. The presence of lymphatic channel invasion or venous invasion was determined by observation of destruction of the lymphovascular wall by tumor cells or the presence of tumor cells within an endothelium-lined space. A positive perineural invasion score was dependent on observation of perineural cancer cells. This analysis was performed on

histological

ment,

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ons stained with hematoxylin and eosin.

Statistical analysis

SPSS software (version 17.0, SPSS Inc., Chicago, IL, USA) was used for this study. To compare variables,  $\chi^2$  analysis was applied. Pearson correlations between

secti-

#### Results

#### Clinical presentation of disease in this cohort

Numerous clinical characteristics of our cohort are described in **Tables 2** and **3**. Our cohort included 116 (62%) male patients and 71 (38%)



**Figure 1.** Graphical depiction of CD44 and CD44v6 expression in Asians with colorectal cancer. The results show the *P* values for correlations observed and illustrate that for most determinants, CD44v6 is superior to CD44.

female patients. Sixty-one (33%) tumors were located in the left colon (defined as descending colon through rectum), 47 (25%) were in the right colon (defined as cecum through transverse colon), and 79 (42%) were in the rectum. Of these cancers, 31 were classified as being protruded CRC, whereas 156 were of the ulcerated type. With respect to the tumor size, 31 (17%) tumors were < 3 cm, 102 (55%) were 3-5 cm, and 54 (28%) were > 5 cm. With respect to the histological characteristics of the cancers involved, 20 (11%) exhibited highly differentiated features, 109 (58%) were classified as being moderately differentiated, and 58 (31%) were poorly differentiated. Staging of the cancers showed that within our cohort, 124 (66%) patients had stage II CRC and 63 (34%) patients had stage III cancer. We concluded that this cohort was suitable for determining the relative usefulness of CD44 versus CD44v6 expression as an indicator for disease prognosis.

## Immunohistochemical detection of CD44v6 and CD44 with regard to poorly differentiated cancer

In normal colorectal mucosa, CD44 is only expressed by a few crypt epithelial cells, but it is present in > 80% of stromal cells. According to the immunohistochemistry results presented in this study, the normal colorectal mucosa expressed small amounts of CD44 and CD44v6. The cells of the villi were negative, whereas crypt cells showed weak and focal expression of CD44 and CD44v6. In addition, weak membranous CD44 expression was observed sparsely in epithelial cells of colon pits in normal colon mucosa. CD44v6 immunoreactivity was also found in some stromal fibroblasts and lymphocytes, in agreement with published data [15]. Subsequently, we analyzed tumor expression of CD44 and CD44v6 in our cohort of CRC patients.

After elimination of cases with incomplete immunostaining or other missing data, we included 5 patients who were  $\leq$  40 years old, 16 patients who were 41-50 years old, 62 patients who were 51-60 years old, 73 patients who were 61-70 years old, and 31 patients who were  $\geq$  72 years old at the time of tumor resection. The median age of patients in this study was 65 years old (range: 31-88 years old). The association between age groups and various morphological variables is presented in **Table 4**.

Within this clearly defined patient group, CD44 expression was detected in 10 (50%) patients with histologically well-differentiated tumors, 68 (62%) patients with intermediate differentiation, and 30 (52%) patients with poorly differentiated tumors. Thus, CD44 expression was not stratified according to differentiation status in this patient cohort ( $\chi^2 = 2.315$ , P > 0.05).

In contrast, when these samples were analyzed for CD44v6 expression, there was a clear correlation between CD44v6 expression and poor



**Figure 2.** Examples of immunostaining. (A1) CD44 and (A2) CD44v6 immunostaining (400× magnification). The immunostaining was localized in the cytoplasm and plasma membrane. These pictures were graded with regard to expression as (+). (B1) CD44 and (B2) CD44v6 immunostaining (400× magnification). The immunostaining was localized in the cytoplasm and cell membrane. These pictures were graded with regard to expression as (++). (C1) CD44 and (C2) CD44v6 immunostaining (400× magnification). The immunostaining was localized in the cytoplasm and cell membrane. These pictures were graded with regard to expression as (++). (C1) CD44 and (C2) CD44v6 immunostaining (400× magnification). The immunostaining was localized in the cytoplasm and plasma membrane. These pictures were graded as (+++). (D) CD44v6 immunostaining (400× magnification). Example of negative staining, although some stromal cells appeared to have a positive signal for CD44v6.

Onlaws stal sources	NI	CD44						
Colorectal cancer	IN	-	+	++	+++			
CD44v6		31	69	84	33			
-	59	18	21	15	5			
+	51	8	25	13	5			
++	72	5	13	47	7			
+++	35	0	10	9	16			
r = 0.407 P = 0.000								

Table 5. Correlation between CD44 and CD44v6 expression in colorectal cancer

 $r_s = 0.407, P = 0.000.$ 

differentiation: 11 (55%) patients with well-differentiated tumors, 75 (69%) patients with intermediate differentiation, and 48 (83%) patients with poorly differentiated tumors were positive for CD44v6 expression ( $\chi^2$  = 6.688, P > 0.05). Hence, in our cohort, CD44v6 was obviously superior to CD44 immunostaining for detecting poorly differentiated tumors (Figures **1** and **2**).

## CD44 and CD44v6 and cancer invasion

Similar results were obtained when CD44 expression was compared to CD44v6 expression with respect to cancer invasion. The expression of CD44 was detected in 19 (49%), 46 (52%), and 43 (72%) patients with tumor invasion into the muscularis, subserosa, and extrasubserosa, respectively ( $\chi^2$  = 7.194, P < 0.05). Thus, CD44 expression correlated with the invasiveness of the CRCs involved. However, this effect was more pronounced when CD44v6 expression was investigated. The expression of CD44v6 was detected in 24 (62%), 52 (59%), and 49 (82%) patients with tumor invasion into the muscularis, subserosa, and extrasubserosa, respectively ( $\chi^2 = 8.830$ , *P* < 0.05).

Analogously, CD44 expression was observed in 62 (50%) patients without angiolymphatic invasion and in 46 (72%) patients with angiolymphatic invasion ( $\chi^2 = 7.952$ , P < 0.05). Meanwhile, CD44v6 expression was observed in 82 (67%) patients without angiolymphatic invasion and in 53 (83%) patients with angiolymphatic invasion ( $\chi^2 = 5.467, P < 0.05$ ).

Finally, CD44v6 expression was superior to CD44 expression with respect to its correlation to metastatic disease. The expression of CD44 was detected in 63 (51%) patients without regional lymph node metastasis, 36 (73%) patients with 1-3 metastases, and 9 (64%) patients who presented with 4 or more metastases. Although CD44 expression correlated with CD44v6 expression in a statistically significant manner ( $\chi^2$  = 7.658, P < 0.05), this effect was more pronounced with regard to the CD44v6 splice variant. The expression of CD44v6 was detected in 82 (66%) patients without regional lymph node metastasis, 41 (84%) patients with 1-3 metastases, and 12 (86%) patients with 4 or more metastases ( $\chi^2$  = 7.220, P < 0.05). Thus, CD44v6 expression shows a better correlation with more aggressive disease as compared to CD44 expression.

# CD44v6 and CD44 expression and cancer staging

In agreement with CD44 and CD44v6 expression indicating cancer aggressiveness, the protein expression levels of both CD44 and CD44v6 were higher in cancerous tissue as compared to the surrounding normal tissue. Of the 187 patients with colon cancer comprising our cohort, CD44 expression was detected in 57.75% (108/187) of the tumor samples. whereas the adjacent normal tissue displayed expression in only 27.3% (51/187) of the samples. This difference was statistically significant  $(x^2 = 35.546, P < 0.05)$ . For CD44v6 expression, this difference in expression between transformed and nontransformed tissue was even more marked: for the 187 patients with colon cancer, CD44v6 expression was detected in 72.19% (135/187) of the tumor samples and in 25.1% (47/187) of the adjacent normal tissue samples ( $\chi^2$  = 82.883, *P* < 0.05) (**Table** 2). Similarly, 108 (58%) colon carcinoma samples were positive for CD44 and 135 (72%) samples were positive for CD44v6. Thus, the expression of CD44v6 was higher than that of CD44 in both stage II and stage III sporadic CRC in our cohort, and the difference was statistically significant ( $\chi^2$  = 4.424, 4.493, P < 0.05) (Table 4). Correlation analysis demonstrated that both CD44 and CD44v6 expression were positively correlated to stage II and stage III sporadic CRC ( $r_s = 0.407, P < 0.05$ ) (Table 5).

For both CD44 and CD44v6 expression, there was no significant difference between the protruded type and ulcerated form of the disease (Table 4). Thus, the difference in expression between CD44 and CD44v6 is mainly related to the aggressiveness of the cancer and not to other cancer subspecies-defining characteristics.

# Discussion

CD44 is an integral cell membrane glycoprotein that is implicated in many functions pertinent to cancer progression. It is known as the principal cell surface receptor for the extracellular matrix glycosaminoglycan hyaluronate. Furthermore, CD44 isoforms that contain heparin sulfate side chains also bind growth factors and promote growth factor receptor-mediated signaling. The intracellular domain of CD44 interacts with certain cytoskeletal proteins, such as ankyrin, merlin, c-src, ezrin, radixin, and moesin [16]. CD44 has multiple activities, including lymphocyte homing, cell adhesion, leukocyte activation, and cell migration. At least 20 variants (v) of CD44 have been reported, which are generated as a consequence of the alternative splicing of the 10 exons (v1-v10) that encode the proximal membrane portion of the extracellular domain [17]. The NH<sub>2</sub>-terminal functional area of CD44 on the cell surface can engage the hyaluronate in the basement membrane of the extracellular matrix, thus regulating the movement and function of cells. By this mechanism, neoplastic cells can adhere to the extracellular matrix and basement membrane of the host cell, resulting in tumor invasion and metastasis. Moreover, degraded products of hyaluronic acid can promote the growth of local vessels, further facilitating invasion and metastasis [18]. Accordingly, CD44 plays an important role in invasion and metastasis of a variety of human cancers, such as breast cancer and prostate cancer [19]. With respect to CRC, the expression levels of CD44 can markedly differ between different cases; but, in general, CD44positive cells show more robust colony formation, higher proliferation, and less spontaneous apoptosis [20].

In the normal colon, members of the CD44 family are only weakly expressed by a few epithelial cells at the base of crypts; however, in this study, we demonstrated that in pericancer normal mucosa 27.3% of such expression can be detected, possibly higher than expected and suggesting that cancer field effects may be involved. It is important to note that the role of CD44 in cancer progression is not unequivocally clear as some studies suggest that CD44 upregulation may be important in tumor invasion and spread, while others implicate that the downregulation or absence of CD44 reflects tumor aggressiveness [7]. In our study, we observed that the expression of CD44 in the colon cancer tissue was substantially higher than that of the pericancer normal mucosa and that CD44 expression correlates with cancer progression and aggressiveness. In apparent agreement, in this study, we observed that CD44 expression was higher in cancer that had metastasized to the lymph nodes than in nonmetastasic cancer. Thus, our results support a role for CD44 upregulation in tumor pathology [21].

Recently, interest in the CD44v6 splice variant of CD44 has grown. So far, CD44v6 has been shown to be a useful prognostic factor for a variety of cancers, including those of the stomach, head and neck, prostate, and lung [22]. In contrast to the standard form of CD44, which is almost ubiquitously expressed, splice variants containing the variant exon v6 are highly restricted in their expression in normal tissues [23]. In the present study, CD44v6 expression in pericancer normal tissue was observed in 25% of the cases, especially in crypt cells with weak and focal expression of CD44v6. Its expression in cancer tissue was 72.19%, which is markedly higher. Other studies have shown by immunohistochemical analysis that CD44v6 expression is related to both colorectal tumor progression and patient survival [4], but such studies have not involved Asian cohorts. Nevertheless, Zahra et al. have demonstrated a significant association between CD44v6 expression and tumor differentiation and the Dukes' stage [24]. Corroborating this effect for an Asian cohort, we observed that CD44v6 expression was associated with poor differentiation, invasion depth, and angiolymphatic invasion. With respect to the clinical significance of CD44v6, Wielenga et al. have reported that CD44v6 expression is largely restricted to the advanced stages of CRC and is more prevalent in metastatic than in nonmetastatic carcinomas [25]. Also, in the present study, we extended this notion by showing that CD44v6 expression was higher in lymph node metastatic cancer than in nonmetastasic cancer. Having been performed with an Asian cohort, our study establishes the universal applicability of CD44v6 as a useful marker for more aggressive disease.

Most importantly, our study demonstrated that positive expression of CD44v6 is higher than that of CD44 in stage II and stage III sporadic CRC (P < 0.05). Thus, CD44v6 expression is superior to CD44 expression with respect to assessing prognosis and is clinically more useful. Therefore, it should be measured in gastroenterological practice instead of CD44.

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#### Disclosure of conflict of interest

#### None.

Address correspondence to: Dr. Lihua Zhao, Department of Pathology, Beijing Civil Aviation Hospital, 1 Gao Jing Jia, Chao Yang District, Beijing 100123, China. Tel: +86 10 85762244-2650; E-mail: dzhao-li@126.com

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