Differential expression of carbohydrate antigen 19-9 in human colorectal cancer: A comparison with colon and rectal cancers

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Abstract. Colorectal cancer is one of the leading causes of cancer-related mortality, being the third most commonly diagnosed cancer among men and the second among women. Accumulating evidence regarding carbohydrate antigen (CA) demonstrated that tumor-associated antigens are clinically useful for the diagnosis, staging and monitoring of human gastrointestinal cancers, particularly colorectal cancer. There has been an extensive investigation for sensitive and specific markers of this disease. Currently, the gastrointestinal cancer-associated carbohydrate antigen 19-9 (CA19-9) is the most widely applied tumor marker in cancer diagnosis. Despite a similar etiology and cancer incidence rates, there are anatomical and clinical differences between colon and rectal cancer, as well as differences regarding tumor progression and adjuvant treatments. To investigate whether CA19-9 is differentially expressed between colon and rectal cancer, we conducted a differential analysis of serum CA19-9 levels among 227 cases of colorectal cancer, analyzing gender, age, Dukes' stage and distant metastasis for human colon and rectal cancer as a single entity, separately and as matched pairs. We demonstrated that the serum CA19-9 levels in colorectal cancer were upregulated in advanced stages with distant metastasis. By contrast, the serum CA19-9 levels in colon cancer displayed a differential and upregulated behavior in advanced stages with distant metastasis. By analyzing as matched pairs, the upregulated serum CA19-9 levels in rectal cancer during the early stages without distant metastasis further supported our hypothesis that the expression of CA19-9 displays a site-specific differential behavior. The integrative analysis suggested a significant difference between human colon and rectal cancer, justifying individualized therapy for these two types of cancer.

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Introduction

Colorectal cancer is one of the leading causes of cancer-related mortality, being the third most commonly diagnosed cancer among men and the second among women (1). The incidence rates of colorectal cancer are rapidly increasing in several areas that were historically at minimal risk, including several countries within Eastern Asia (2). Early diagnosis and intervention may be crucial in improving therapeutic effectiveness and prolonging survival time. In the initial stage of tumorigenesis, tumor markers are widely used for diagnosis, staging, and monitoring of colorectal cancer in clinical laboratory tests (3). These markers are usually proteins released from dying tumor cells or produced by neoplastic cells. Certain specific proteins are expressed only in tumor cells and are useful for the detection and diagnosis of specific malignant tumors. Non-specific proteins or markers associated with malignant tumor cells are oncofetal or carcinogenic antigens, such as carcinoembryonic antigen (CEA), α-fetoprotein, carbohydrate antigen 125, carbohydrate antigen 19-9 (CA19-9), tissue plasminogen activator and tissue polypeptide-specific antigen (4).

Koprowski *et al* (5) described CA19-9 as a monoclonal antibody in 1979. Since then, CA19-9 has been increasingly used to detect serum antigens associated with specific malignancies. It was previously demonstrated that CA19-9 is produced by adenocarcinomas of the pancreas, stomach, gallbladder, colon, ovary and lung and is released into the circulation. Elevated serum CA19-9 levels have been associated with a range of gastrointestinal malignant tumors, including colorectal carcinoma. CA19-9 may also be useful in determining the nature of pancreatic masses (6). Tumor markers are most useful for monitoring the response to therapy and detecting early relapses. Each tumor marker has a variable range of application for screening, determining diagnosis and prognosis, assessing the response to therapy and monitoring

cancer recurrence. CEA is most frequently used to detect gastrointestinal malignant tumors and the variation of CEA values reflects individual response to clinical therapy (7). By contrast, in the screening of gastrointestinal malignancies, the American Society of Clinical Oncology guidelines suggested that serum testing for CA19-9 is an integral part of the diagnosis and management of colorectal carcinomas (8). Numerous studies addressed the potential utility of CA19-9 assessment in adenocarcinomas of the colon and rectum (9). The reported incidence of elevated serum CA19-9 levels in colorectal cancer ranges from 20-40% (10). The incidence of elevated CA19-9 levels is stage-related, with the highest sensitivity observed in patients with metastases. However, the sensitivity of CA19-9 has always been lower compared to that of the CEA for all the stages of this disease. The false-positive rate is 15-30% in patients with non-neoplastic diseases of the pancreas, liver and biliary tract. Consequently, CA19-9 may not be used for screening asymptomatic populations (11,12). Serum screening tests require sufficient specificity and high sensitivity to detect early-stage carcinoma. Individuals who undergo serum tests display varying results. Benign conditions, such as cirrhosis, cholestasis, cholangitis and pancreatitis, may also result in an elevation of the CA19-9 levels. False-positive results prompt the research for more specific and sensitive tumor markers.

Colorectal cancer is a major contributor to cancer-related mortality and morbidity (1). The diagnosis and therapy of colon and rectal cancer as a single entity has attracted considerable attention. Although these two types of cancer have similar etiology, incidence rates, surgical and radiotherapeutic management implications, accumulating evidence reveals notable differences. The differences between the colon and rectum are largely anatomical and biological and may affect prognosis. Cancers of the colon and rectum may develop differently due to their distinctive embryological origin (midgut/hindgut and hindgut, respectively) and differential exposure to bowel content. Furthermore, colon and rectal cancers have differences regarding anatomy and blood circulation. Venous blood from the colon flows to the liver via the portal vein, whereas rectal venous blood partially bypasses the liver. Blood circulation often affects tumor relapse. Rectal cancers exhibit higher rates of localized regional relapse and lung metastases, whereas colon cancers have a higher tropism for liver spread (13). The serum concentration of tumor markers may be affected by metabolism in the liver. This may explain the differential expression of CA19-9 between these two malignancies. There is also a difference in clinical presentation, prognosis and, possibly, in genetic and environmental epidemiology (14). The differential behavior of single molecules in colon and rectal tumors may help elucidate the molecular basis of these two types of cancer and their prognostic and therapeutic implications (15). Despite clinical evidence of the differences between colon and rectal cancer, the number of studies that have addressed the molecular differences between the two diseases is limited. Through the analysis of several molecular markers, Kapitejin *et al* (16) demonstrated a significantly different β-catenin and p53 expression between colon and rectal cancers and concluded that these two malignancies may follow different mechanisms of oncogenesis (17). Furthermore, the analysis of KRAS mutations revealed that they are more specifically detectable in colon compared to rectal cancer (18). As regards epidemiological, morphological and molecular characteristics, the mechanisms of colorectal carcinogenesis may differ according to tumor location. It was suggested that a mechanism exists that promotes the progression of mucosal lesions to invasive cancers in the colon and rectum (19). Therefore, we decided to investigate whether there are differences in the serum levels of CA19-9 between patients with colon and those with rectal cancer.

In our study, a differential analysis of serum CA19-9 levels according to gender, age, Dukes' stage and distant metastasis for human colon and rectal cancer was conducted. As a significant predictor for colorectal cancer invasion and metastasis, serum CA19-9 levels in colon cancer displayed a notable upregulated behavior in advanced stages of the tumor with distant metastasis. By contrast, the upregulated serum CA19-9 levels in the early stages of rectal cancer without distant metastasis further supported our hypothesis that the expression of CA19-9 displayed a site-specific differential behavior. The integrative analysis suggested a significant difference between colon and rectal cancer and also indicated an important role for CA19-9 in early diagnosis and individualized therapy of human colorectal cancer.

Materials and methods

Patient specimens. Preoperative serum samples were obtained from 227 patients (135 men and 92 women) with histologically verified colorectal cancer. The patients were classified into the younger group (<60 years old, 90 cases) and the elder group (≥60 years old, 137 cases). As the focus of our study, 116 colon and 111 rectal cancers were staged according to the modified Dukes' classification (stage A, 54; stage B, 51; stage C, 57; and stage D, 65 cases). As mentioned above, 116 patients had colon cancer (30 patients had stage A, 28 stage B, 28 stage C and 30 stage D) and 111 patients had rectal cancer (24 patients had stage A, 23 stage B, 29 stage C and 35 stage D). According to our statistics, specimens with Dukes' stage A, B and C colorectal cancer did not exhibit significant differences. However, patients with Dukes' stage D disease were quite different. Therefore, patients with Dukes'stages A-C were considered as having early-stage disease, whereas those with Dukes' stage D were considered as having advanced-stage disease. Patients in the distant metastasis group had either lymph node or distant metastases. The CA19-9 values were obtained from the serum of the patients who underwent surgical resection at the First and Second Affiliated Hospitals of Dalian Medical University beteween 2010 and 2012.

Serum collection and CA19-9 assay. The preoperative serum samples were obtained prior to the administration of radiation treatment or chemotherapy. Blood samples were collected, separated by centrifugation and the serum samples were stored at -20˚C until the assays were performed. The CA19-9 kit was provided by Diagnostic Products Corporation (DPC, Tianjin, China). The serum CA19-9 levels were determined by the DPC Gamma C12 immunoradiometric gamma counter (DPC). Data on patient specimens were furnished by the First and Second Affiliated Hospitals of Dalian Medical University between 2010 and 2012.

Table I. Comparison of serum CA19-9 levels in colorectal cancer among different groups.

Pathological characteristics	No.	Median CA19-9 (U/ml)	P-value
Gender			0.615
Male	135	15.30	
Female	92	13.48	
Age (years)			0.665
<60	90	14.65	
>60	137	14.20	
Dukes' stage			0.005[a]
Early	162	12.75	
Advanced	65	18.64	
Distant metastasis			0.016[a]
-	161	12.70	
+	66	18.06	

[a]Statistically significant. CA19-9, carbohydrate antigen 19-9.

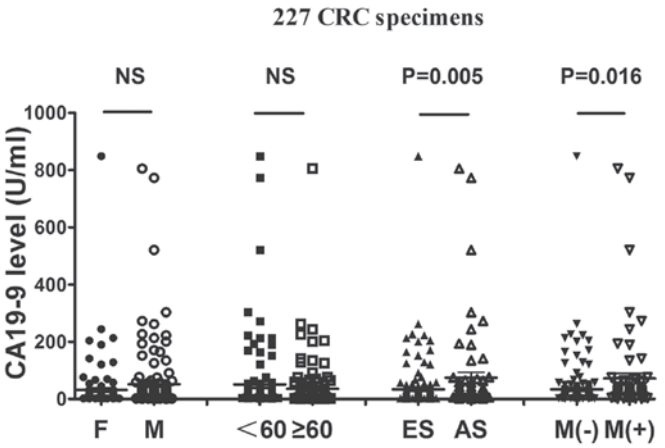

Figure 1. Different mean values of carbohydrate antigen 19-9 (CA19-9) among the 227 CRC specimens according to different groups. The 227 CRC specimens were divided into 4 groups according to gender, age (<60 and ≥60 years), Dukes' stage and metastasis. Although patients with different gender and age, exhibited no statistically significant differences, the differences between patients with early- and advanced-stage disease (P=0.005) and between patients with and those without metastasis (P=0.016) were statistically significant. The results indicated that high CA19-9 values were more significant for patients with Dukes' stage D disease or with metastasis. Furthermore, the expression of CA19-9 was increased during tumorigenesis. CRC, colorectal cancer; F, female; M, male; <60, patients younger than 60 years; ≥60, patients older than 60 years; ES, early stage (Dukes' A, B and C); AS, advanced stage (Dukes' D); M(-), patients without metastasis; M(+), patients with metastasis; NS, difference without statistical significance.

Statistical analysis. A differential analysis of the 227 samples according to serum CA19-9 levels, gender, age, Dukes' stage and metastasis was separately conducted for human colon and rectal cancer. The serum levels of CA19-9 did not follow a normal distribution and the significance between the groups was calculated by non-parametric statistical methods (Mann-Whitney and Kruskall-Wallis tests). The centralized tendency of each group was described by geometric mean due to the right skewness of the frequency distribution. The statistical analysis was performed with SPSS software for Windows, version 11.5 (SPSS Inc., Chicago, IL, USA). Our results were accurate to four digits. P<0.05 was considered to indicate a statistically significant difference.

Results

Upregulated serum CA19-9 levels in colorectal cancer with advanced stage and distant metastasis. A total of 227 colorectal cancer patients, including 116 colon and 111 rectal cancer cases, were clinically diagnosed by imaging and histopathology. For the mean values (mean ± SEM) of the CA19-9 levels, there was no statistical difference between the sera collected from colon cancer (45.85±11.05 U/ml) and those collected from rectal cancer patients (44.88±9.150 U/ml) (P=0.9467). Therefore, we analyzed them first as a single entity (Table I and Fig. 1). We observed that the serum CA19-9 levels between the gender groups (135 men and 92 women) and the age groups (90 patients included in the younger and 137 patients in the elder group) exhibited no statistically significant difference (P>0.05). However, the mean values of serum CA19-9 levels exhibited a significant correlation with Dukes' stage (P=0.005) and distant metastasis (P=0.016). The mean values of the serum CA19-9 levels in patients with advanced-stage disease (74.30±11.29 U/ml) and distant metastasis (71.33±18.49 U/ml) were more upregulated compared to those in patients with early-stage disease (33.77±6.284 U/ml) and without distant metastasis (33.86±6.322 U/ml). The expression of CA19-9 exhibited a tendency for increase, suggesting that the mean values of serum CA19-9 levels reflected tumor progression and were a significant predictor for colorectal carcinoma invasion and metastasis (Table I and Fig. 1). This finding may be associated with the function of CA19-9 in the differentiation and migration of tumor cells.

Upregulated serum CA19-9 levels in colon cancer, but not in rectal cancer, with advanced stage and distant metastasis. A number of studies indicated the differences between colon and rectal cancer (14,16,21,24); therefore, we conducted further separate analyses of the serum CA19-9 levels in colon and rectal cancer (Tables II and III, Fig. 2). In colon cancer specimens, the results demonstrated that the serum CA19-9 levels in colon cancer were significantly correlated with Dukes' tumor stage (P=0.002) and distant metastasis (P=0.003). The upregulated gradient of CA19-9 levels was quite notable. The mean CA19-9 values in patients with advanced-stage disease (113.7±25.32 U/ml) and distant metastasis (111.1±25.03 U/ml) were significantly higher compared to those in patients with early-stage disease (19.86±2.468 U/ml) and without distant metastasis (19.77±2.481 U/ml). Similar to the holistic analyses of colorectal cancer, the differences in colon cancer specimens between gender and age groups were not statistically significant. We confirmed that CA19-9 expression in colon cancer patients was significantly upregulated during the process of tumorigenesis and metastasis (Table II and Fig. 2A). However, the mean values of serum CA19-9 levels in rectal cancer displayed no statistically significant difference among any of the 4 groups (Table III and Fig. 2B). This finding indicated

Table II. Comparison of serum CA19-9 levels in colon cancer among different groups.

Pathological characteristics	No.	Median CA19-9 (U/ml)	P-value
Gender			0.960
Male	68	12.55	
Female	48	12.35	
Age (years)			0.522
<60	42	10.03	
>60	74	13.28	
Dukes' stage			0.002[a]
Early	86	11.38	
Advanced	30	28.99	
Distant metastasis			0.003[a]
-	84	11.38	
+	32	26.38	

[a]Statistically significant. CA19-9, carbohydrate antigen 19-9.

Table III. Comparison of serum CA19-9 levels in rectal cancer among different groups.

Pathological characteristics	No.	Median CA19-9 (U/ml)	P-value
Gender			0.583
Male	67	17.11	
Female	44	14.77	
Age (years)			0.270
<60	48	16.98	
>60	63	15.30	
Dukes' stage			0.570
Early	76	15.21	
Advanced	35	17.35	
Distant metastasis			0.452
-	77	15.11	
+	34	17.41	

CA19-9, carbohydrate antigen 19-9.

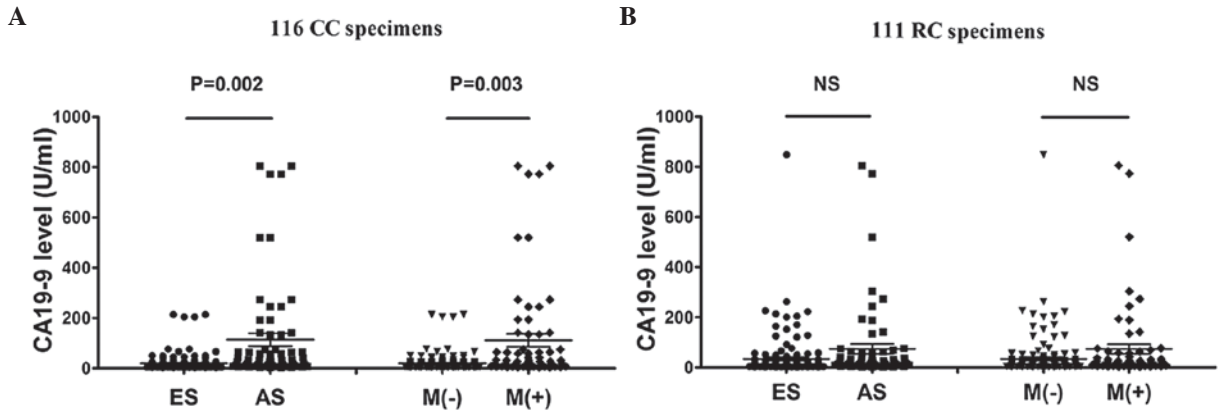

Figure 2. Different mean values of carbohydrate antigen 19-9 (CA19-9) among the 116 CC and the 111 RC specimens separately. The 227 CRC specimens included 116 CC and 111 RC patients. The 116 CC and 111 RC specimens were separately divided into 4 groups according to gender, age (<60 and ≥60 years), Dukes' stage and metastasis. (A) The differences between early- and advanced-stage disease (P=0.002) and the differences between patients with and those without metastasis (P=0.003) in the 116 CC specimens were statistically significant. There were no significant differences regarding the remaining two variables. (B) Among the 111 RC patients divided into the 4 different groups, there were no statistically significant differences in the mean CA19-9 values. CC, colon cancer; RC, rectal cancer; CRC, colorectal cancer; <60, patients younger than 60 years; ≥60, patients aged 60 years or older; ES, early stage (Dukes' A, B and C); AS, advanced stage (Dukes' D); M(-), patients without metastasis; M(+), patients with metastasis; NS, difference without statistical significance.

that CA19-9 expression was not only associated with invasion and metastasis, but also varied with tumor location. The mean CA19-9 values in patients with advanced-stage disease (74.30±19.27 U/ml) and distant metastasis (73.48±19.00 U/ml) were significantly higher compared to those in patients with early-stage disease (33.75±6.284 U/ml) and without distant metastasis (33.86±6.322 U/ml).

Differential expression of serum CA19-9 levels between colon and rectal cancer in early-stage disease without distant metastasis. Previous studies indicated that colon and rectal cancer were similar but different types of cancer (14,16,24,26). Therefore, we continued our study by analyzing colon and rectal cancer as matched pairs (Table IV and Fig. 3). According to our statistical data, the mean values of serum CA19-9 levels in early-stage disease (P=0.015) and without distant metastasis (P=0.021) were significantly more upregulated in rectal compared to colon cancer, with a statistically significant difference (Table IV and Fig. 3B). There was no distinction between colon and rectal cancer in the cohort with gender and age groups (Table IV and Fig. 3A). However, the serum CA19-9 levels in rectal cancer patients with early-stage disease (76 patients) was significantly more upregulated compared to those in colon cancer patients with the same Dukes' stage (86 patients) (P=0.015). In addition, the CA19-9 levels in rectal cancer patients without distant metastasis (including 84 colon cancer and 77 rectal cancer patients) was also statistically more upregulated compared to those in colon cancer patients in the same matched pairs (P=0.021) (Table IV

Table IV. Comparison of serum carbohydrate antigen 19-9 (CA19-9) levels between colon and rectal cancer.

Pathological characteristics	Cases		Median CA19-9 (U/ml)		P-value
	Colon cancer	Rectal cancer	Colon cancer	Rectal cancer	
Gender					
Male	68	67	12.55	17.11	0.165
Female	48	44	12.35	14.77	0.298
Age (years)					
<60	42	48	10.03	16.98	0.075
>60	74	63	13.28	15.30	0.433
Dukes' stage					
Early	86	76	11.38	15.21	0.015[a]
Advanced	30	35	28.99	17.35	0.231
Distant metastasis					
-	84	77	11.38	15.11	0.021[a]
+	32	34	26.38	17.41	0.346

[a]Statistically significant. CA19-9, carbohydrate antigen 19-9.

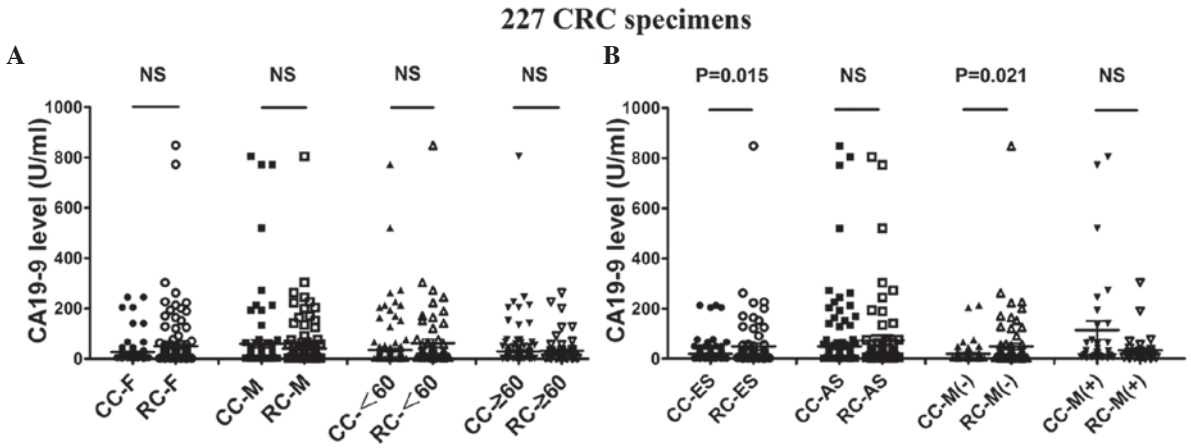

Figure 3. Different mean values of carbohydrate antigen 19-9 (CA19-9) between CC and RC patients according to gender, age, Dukes' stage and metastasis. A total of 227 CRC patients, including 116 CC and 111 RC patients, were divided into 4 groups according to gender, age (<60 and ≥60 years), Dukes' stage and metastasis. Furthermore, CA19-9 values were compared between CC and RC as different aspects of tumor location. Among the patients of different gender and age, female RC and male CC patients exhibited a tendency for high CA19-9 levels. Of note, the two age groups exhibited high mean CA19-9 values in RC patients. However, there were no significant differences in the mean CA19-9 values between gender and age difference groups (A) Among the patients with advanced Dukes' stage and metastasis, although CC patients exhibited higher CA19-9 levels compared to RC patients during the same period, the differences were not statistically significant. Among these 4 groups, we demonstrated that RC patients with early-stage disease (P=0.0015) and without metastasis (P=0.0021) exhibited statistically significant higher mean CA19-9 values compared to CC patients. CC, colon cancer; RC, rectal cancer; CRC, colorectal cancer; F, female; M, male; <60, patients younger than 60 years; ≥60, patients aged 60 years or older; ES, early stage (Dukes' A, B and C); AS, advanced stage (Dukes' D); M(-), patients without metastasis; M(+), patients with metastasis; NS, difference without statistical significance.

and Fig. 3B). These results demonstrated that the expression of CA19-9 displayed a site-specific differential behavior. Moreover, they suggested that serum CA19-9 levels may be more sensitive in the early diagnosis of rectal cancer. The differential expression of CA19-9 during the early stages of tumorigenesis also indicates the distinction between colon and rectal carcinomas.

Discussion

Similar to other tumor-associated antigens, it appears that elevated serum CA19-9 levels are associated with gastrointestinal malignancies, particularly advanced colorectal cancer. Of more relevance to the potential use of CA19-9 as a screening test is the comparison of CA19-9 serum levels between individuals with colon and rectal cancer. To identify possible biological differences between colon and rectal tumors, we conducted a differential analysis of 227 specimens, analyzing serum CA19-9 levels according to gender, age, Dukes' stage and distant metastasis in human colon and rectal cancer. We demonstrated that the serum CA19-9 levels in colorectal cancer of advanced stage and with distant metastasis were significantly upregulated, suggesting that the

expression of CA19-9 reflects tumor invasion and metastasis. Correspondingly, we confirmed that serum CA19-9 levels displayed a notable upregulation in colon cancer specimens of advanced stage and with distant metastasis. However, we failed to demonstrate this upregulation of CA19-9 in rectal cancer specimens, suggesting that colon and rectal cancer are similar but different types of cancer. In our continued comparison, by analyzing colon and rectal cancer as matched pairs, the serum CA19-9 levels in early-stage disease and without distant metastasis exhibited statistically more significant upregulation in rectal compared to colon cancer. It was evident that there was no distinction between colon and rectal cancer in the cohort of gender and age groups. These results further supported our hypothesis that the expression of CA19-9 displayed a site-specific behavior.

The issue of whether colon and rectal cancer should be considered as a single entity or two distinct entities is still debated upon. There are differences between colon and rectal cancer with respect to patient gender and age, as well as tumor progression and adjuvant treatments (17). Despite a similar etiology and cancer incidence rates, the anatomical and clinical distinction should not be overlooked. A previous study attempted to identify and characterize the genetic changes involved in the colorectal malignant transformation process (18). Through the analysis of several molecular markers, Kapitejin *et al* demonstrated a significantly different β-catenin and p53 expression between colon and rectal cancers and concluded that these two types of cancer may follow separate mechanisms of oncogenesis (16). Furthermore, several critical genes and pathways have been shown to be involved in the initiation and progression of colorectal cancer. Large-scale sequencing analyses identified numerous recurrently mutated genes and a recurrent chromosomal translocation (20-22). These include the cyclin A2, COX2, RAS-MAPK, PI3K, TGF-β, p53 and DNA mismatch-repair pathways (22). Moreover, colon cancers exhibit a higher number of mutations, including KRAS and BRAF mutations (15). The CIN pathway is far more common in rectal compared to colon cancers (24,25). In addition, several homeobox genes were found to be associated with tumor location (26). A different number of mutations induce varying mechanisms of oncogenesis in colon and rectal cancer. The site-specific differential behavior of CA19-9 in colon and rectal carcinoma demonstrated a different tumor identification and progression.

It was previously demonstrated that the serum CA19-9 levels were the most significant prognostic indicator of patients with metastatic colorectal cancer (27). Our results, although similar, differed in detail. The serum CA19-9 levels were significantly upregulated in association with advanced-stage disease and distant metastasis in colon cancer, but not in rectal cancer. In our continued study, serum CA19-9 levels in early-stage disease and without distant metastasis were statistically significantly more upregulated in rectal cancer compared to colon cancer, suggesting that CA19-9 is more sensitive for early diagnosis of rectal cancer. The differential expression of CA19-9 between colon and rectal cancer further supports that colon and rectal cancer are similar but different types of cancer.

In conclusion, our study strongly suggests that the expression of CA19-9 displays a site-specific differential behavior. The internal mechanism underlying our results has not been elucidated, although a consistent difference was observed between colon and rectal cancer. This observation indicates an important role for CA19-9 in early diagnosis and tumor metastasis. In our future study, we aim to recommend the individualization of treatment for human colon and rectal cancer.

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