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Original Article

Expression and clinical significance of serum MACC1 in patients with colorectal cancer

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Abstract: Objective: In this study, we investigated the serum expression and clinical significance of metastasis-associated in colon cancer-1 (MACC1) in colorectal cancer patients. Methods: We retrospectively analyzed serum samples from 206 colorectal cancer patients who received treatment in our hospital and from 141 healthy individuals (control group) between August 2011 and August 2012. MACC1 expression was assessed using ELISA. The correlation between MACC1 expression and clinical characteristics of colorectal cancer was analyzed using SPSS statistical software. Results: The serum expression of MACC1 in colorectal cancer patients was significantly higher than in healthy controls ($p < 0.05$). We also stratified patient groups by their risk level. In colorectal cancer patients, serum expression of MACC1 was closely associated with serum CA19-9 and CEA level, lymph node and distant metastasis, neural invasion, tumor deposit, and higher TNM stage. However, no significant correlation was found between MACC1 expression and age, family history, tumor location, depth of tumor invasion, vascular tumor thrombus, or pathologic type ($p > 0.05$). In addition, the overall survival rate in patients with high MACC1 expression was significantly lower than in those with low expression ($\chi^2 = 12.158$, $p = 0.001$). Conclusions: Serum MACC1 expression was increased in patients with colorectal cancer and was significantly related to malignant evolution and clinical prognosis of colorectal cancer. MACC1 may be a good serological marker for diagnosing and predicting the prognosis of colorectal cancer.

Keywords: MACC1, colorectal cancer, serum, prognosis

Introduction

Colorectal cancer is a common malignancy of the digestive system. Its morbidity and mortality rate ranks third among common tumors [1]. The early clinical symptoms of colorectal cancer are usually occult on onset and may be ignored or missed; as such, a high number of patients are diagnosed at later stages. Treatment failure and death are primarily caused by metastasis and recurrence. Early detection and standardized diagnosis and treatment are crucial for improving the survival of patients with colorectal cancer. Therefore, determining specific indices, such as serum markers, for early diagnosis is important.

Metastasis-associated in colon cancer-1 (MACC1) is an oncogene recently discovered by Stein in 2009 [2]. Located on chromosome 7 (7p21.1), it is a key regulator in the HGF/Met signaling pathway and plays an important role in tumor metastasis and invasion. Follow-up studies have shown that MACC1 overexpression is closely related to the metastatic and invasive capabilities of many malignancies, including colon [3, 4], gastric [5-7], pancreatic [8], and liver cancers [6, 9], among others. Previous studies have shown that MACC1 can be used as an independent predictor of tumor development [10]. In our previous research, we examined the serum expression of MACC1 in pancreatic cancer patients and found that MACC1 can be used as a sensitive indicator for the early diagnosis of pancreatic cancer and patient prognosis [8]. However, such studies on MACC1 in colorectal cancer are yet to be conducted. Therefore, in this study, we evaluated the serum expression of MACC1 in colorectal cancer patients, analyzed the correlation

Table 1. Correlation between serum MACC1 expression levels and clinicopathological parameters of colorectal cancer

Clinicopathological parameters	No. of cases	MACC1 expression				χ^2	*P*-value
		Low	(%)	High	(%)		
Gender						9.042	0.003
Female	65	34	52.3	31	47.7		
Male	141	43	30.5	98	69.5		
Age (years)						2.746	0.097
≤ 60	101	32	31.7	69	68.3		
> 60	105	45	42.9	60	57.1		
Smoking status						0.054	0.816
No	129	49	38.0	80	62.0		
Yes	77	28	36.4	49	63.6		
Drinking status						0.001	0.992
No	131	49	37.4	82	62.6		
Yes	75	28	37.3	47	62.7		
Family history						0.777	0.378
No	159	62	39.0	97	61.0		
Yes	47	15	31.9	32	68.1		
HBV infection						0.023	0.879
No	181	68	37.6	113	62.4		
Yes	25	9	36.0	16	64.0		
CEA						2.345	0.126
< 5 ng/ml	117	49	41.9	68	58.1		
≥ 5 ng/ml	89	28	31.5	61	68.5		
CA19-9						5.080	0.024
< 37 U/ml	159	66	41.5	93	58.5		
≥ 37 U/ml	47	11	23.4	36	76.6		
Ferrtin						0.118	0.731
< 274 ng/ml	183	68	37.2	115	62.8		
≥ 274 ng/ml	22	9	40.9	13	59.1		
Primary tumor location						2.102	0.350
Left	30	14	46.7	16	53.3		
Right	46	19	41.3	27	58.7		
Rectum	130	44	33.8	86	66.2		
Invasion depth						0.520	0.471
T1+T2	53	22	41.5	31	58.5		
T3+T4	153	55	35.9	98	64.1		
Lymph node metastasis						25.292	0.001
N0	107	57	53.3	50	46.7		
N1	56	14	25.0	42	75.0		
N2	43	6	14.0	37	86.0		
Distant metastasis						14.054	0.003
M0	164	71	43.3	93	56.7		
M1a	25	5	20.0	20	80.0		
M1b	14	0	0.0	14	100.0		
M1c	3	1	33.3	2	66.7		
Vascular invasion						0.670	0.413
No	168	65	38.7	103	61.3		

between MACC1 expression and clinicopathological characteristics of colorectal cancer, and explored the relationship between MACC1 expression and disease prognosis.

Materials and methods

Patients

We assayed preoperative serum samples from 206 patients with colorectal cancer (141 men, 65 women; average age 60.12 ± 11.06 years) and from 141 healthy controls (average age: 58.48 ± 10.82 years) between August 2011 and August 2012 from Zhejiang Cancer Hospital Biospecimen Repository. Patients were selected using the following inclusion criteria: (1) Written informed consent was provided, (2) Pathologically diagnosed colon cancer, and (3) No preoperative neoadjuvant treatments, such as radiotherapy or chemotherapy. No significant differences in age and sex were noted between the two groups ($p > 0.05$). Tumor staging was according to the 2017 colorectal cancer staging criteria (8th edition) of the American Joint Committee on Cancer.

ELISA

Approximately 5 mL of venous blood was collected from patients with colorectal cancer, coagulated at room temperature, and centrifuged at 3000 rpm for 20 min, and the supernatant (serum) was collected. Subsequently, 200 μL of serum was aliquoted into tubes and stored at -80°C

Yes	38	12	31.6	26	68.4		
Nerve invasion						8.359	0.004
No	150	65	43.3	85	56.7		
Yes	56	12	21.4	44	78.6		
Tumor deposit						7.319	0.007
No	158	67	42.4	91	57.6		
Yes	48	10	20.8	38	79.2		
Pathological type						1.633	0.201
Adenocarcinoma	192	74	38.5	118	61.5		
Mucous adenocarcinoma	14	3	21.4	11	78.6		
Differentiation						3.211	0.200
Low	45	14	31.1	31	68.9		
Middle	157	60	38.2	97	61.8		
High	4	3	75.0	1	25.0		
TNM stages						25.085	0.001
I+II	98	54	55.1	44	44.9		
III+IV	108	23	21.3	85	78.7		

MACC1, metastasis-associated in colon cancer-1.

for centralized detection. The serum specimens were gradually thawed to room temperature before use. MACC1 serum levels were measured via ELISA (Uscn Life Science Inc. Wuhan, China) according to the manufacturer's instruction, and all experiments were carried out in duplicate. A total of 100 μL each of standard (provided by the manufacturer), sample, and blank specimens were added to a 96-well plate that had been coated with MACC1 antibody and the plate was incubated for 2 hours at 37°C. Each well was aspirated and subsequently washed with a wash buffer (provided by the manufacturer). Then, 100 μL of Detection Reagent A was added to each well, and the plate was incubated for another hour at 37°C. After removing any unbound antibody, 100 μL of Detection Reagent B was added to each well, followed by incubation for 30 minutes at 37°C. After washing, 90 μL of substrate solution was added to the wells. The plate was incubated for 20 minutes at 37°C in the dark. The reaction was then terminated using 50 μL of Stop Solution. The optical density of each well was determined using a microplate reader at 450 nm. The MACC1 concentrations of the samples were determined using standard curves generated using a two-parameter logistic curve fit analysis.

Assessment criteria

The optimal diagnostic cut-off value was determined by calculating the Youden index of the receiver operating characteristic (ROC) curve. Samples with values above this threshold were defined as having high MACC1 expression.

Follow-up

Overall survival (OS) was defined as the time from the date of diagnosis to death or last follow-up, which was on January 20, 2017. Data for patients lost to follow-up were censored.

Statistical analysis

Statistical analyses were performed using SPSS (version 18.0, IBM Corporation, Armonk, NY, USA) and GraphPad Prism (version 5; GraphPad Software, La Jolla, CA, USA) software. The measurement data are presented herein as mean ± SD, and were statistically analyzed using Mann-Whitney U test or Wilcoxon test. Receiver operating characteristics (ROC) analysis was operated to determine serum MACC1 sensitivity and specificity in distinguishing colorectal cancer patients from healthy controls. Sensitivity, specificity and area under the ROC curve (AUC) were performed to evaluate the diagnostic value of MACC1 expression. The score closest to the value under peak sensitivity and specificity was defined as cut-off value. The numerical data were statistically analyzed using chi-squared test or Fisher's exact test. The level of statistical significance was set at 0.05. The Kaplan-Meier estimate was used for univariate survival analysis and for drawing the survival curves. COX regression analysis was used for univariate and multivariate analysis of correlation between clinicopathological variables and overall survival.

Results

Characteristics of included patients

Our study included 206 colorectal cancer patients and 141 healthy controls. The clinical information of these patients is provided in **Table 1**. In these cases, 89 patients (about

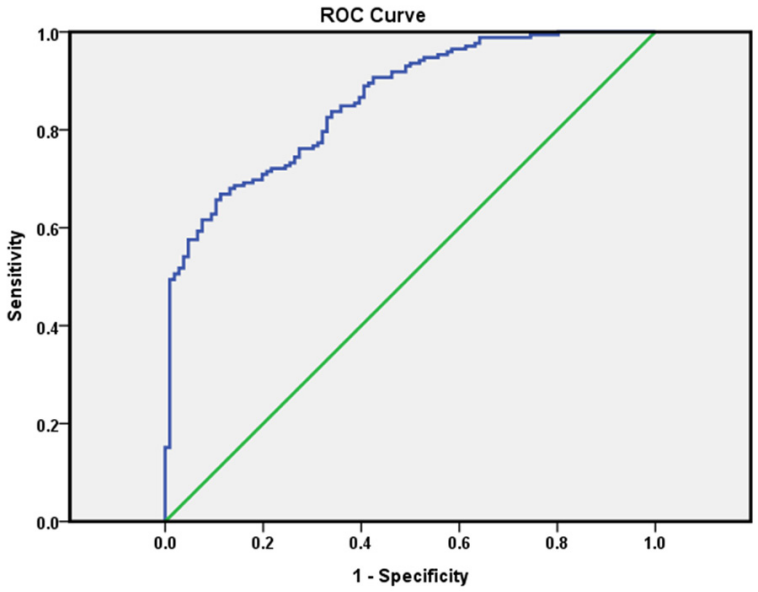

Figure 1. Receiver operating characteristic (ROC) analysis was performed to determine the sensitivity and specificity of circulating MACC1 protein level using area under the ROC curve (AUC) analysis. AUC = 0.859 (0.817-0.902); Sensitivity = 0.669; Specificity = 0.887; Cut-off value = 2.79 ng/mL.

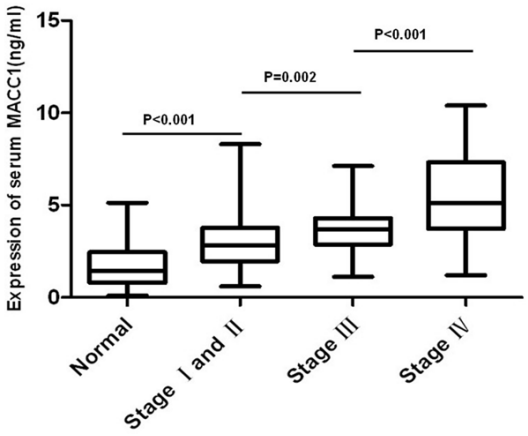

Figure 2. Association between serum MACC1 expression and clinicopathological variables. Comparison of serum MACC1 expression between healthy controls and different stage CRC patients ($p < 0.001$).

43.2%) were diagnosed as high CEA level (> 5 ng/ml) and 47 patients (about 22.8%) were diagnosed as high CA19-9 level (> 37 U/ml). Of those with cancer, 76 had colon cancer (36.9%) and 130 had rectal cancer (63.1%). Lymph node metastases were presenting 99 cases (48.1%), while metastasis patients accounted for about 20.4% (42/206). Low-Middle differentiated colorectal cancer was about 98.1% of CRC pathological types and well differentiated colorectal carcinoma is only 2%. The positive rate of vascular invasion in the 206 tumor tissues was 18.4% (38/206) and the positive rate of never invasion was 27.2% (56/206).

Serum MACC1 expression in patients with colorectal cancer

The area under the ROC curve of serum MACC1 expression in patients with colorectal cancer was 0.859 (95% confidence interval: 0.817-0.902; $p < 0.001$) (**Figure 1**). As the Youden index is calculated by adding the sensitivity and specificity and subtracting 1, we found that the Youden Index reached the maximum value when MACC1 expression was 2.79 ng/mL. This was used as the optimal diagnostic cut-off value for our study, that is, if MACC1 concentration was greater than 2.79 ng/ml, then MACC1 expression was regarded as high. Of the serum specimens from 98 patients with early-stage colorectal cancer, 44 (44.9%) had high MACC1 expression. Meanwhile, of the serum specimens from 108 patients with advanced colorectal cancer, 85 (78.7%) had high MACC1 expression. By contrast, only 12 (8.5%) of the 141 healthy controls showed high MACC1 expression. As shown in **Figure 2**, MACC1 expression in patients with colorectal cancer was significantly higher than that in healthy controls ($\chi^2 = 125.94$, $p < 0.001$).

Correlation between serum MACC1 levels and clinicopathological characteristics of patients with colorectal cancer

As shown in **Table 1**, the results of correlation analysis between serum MACC1 levels and clinicopathological data of our patients did not show any significant correlations between MACC1 expression and age, smoking history, family history, drinking history, history of HBV infection, carcinoembryonic antigen (CEA) expression, ferritin levels, tumor location, local infiltration depth, vascular invasion, CEA expressions, pathology type, or the degree of pathological differentiation ($p > 0.05$). However,

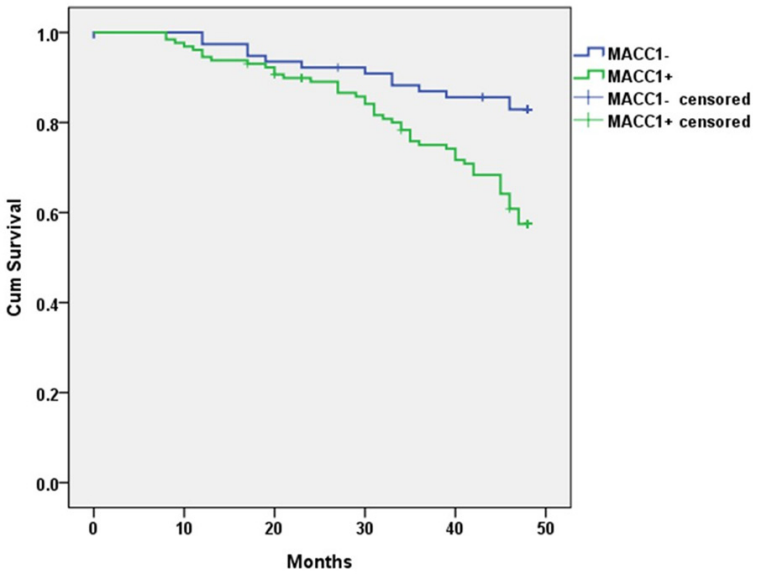

Figure 3. Overall Survival of CRC patients based on serum levels of MACC1. CRC patients with high serum expression of MACC1 demonstrated shorter survival when compared with patients with low MACC1 levels (X^2 = 12.158, p = 0.001).

we found that CA19-9 expressions were significantly correlated with high MACC1 expression (p = 0.024). Similar to the results we found in pancreatic cancer[7], MACC1 expression was significantly associated with lymph node metastasis, distant metastasis, and late tumor stage (p < 0.001, p = 0.003, p < 0.001, respectively). In addition, we found that high MACC1 expression were closely correlated with neural invasion and tumor deposit formation (p = *0.004* and p = 0.007, respectively).

Association between serum MACC1 expression and the prognosis of colorectal cancer patients

Among the 206 patients with colorectal cancer in the present study, 65 died and 14 were lost to follow-up at the end of January 2017. Kaplan-Meier survival analysis showed that the OS of patients with high MACC1 expression was shorter than that of patients with low expression, and the difference was statistically significant (χ^2 = 12.158, p = 0.001; **Figure 3**). Besides, the cox survival analysis was used to analyze the prognostic factors for the 206 CRC patients. The value of various risk factors in predicting colorectal cancer patient prognosis was shown in **Table 2**.

Discussion

With the changes in standards of living and diet, the incidence of colorectal cancer is also increasing yearly. Its high heterogeneity, complex pathogenesis, and likely recurrence and metastasis make it a serious threat to human health and safety. Therefore, early diagnosis and effective control of metastasis are extremely important in colorectal cancer. Currently, colorectal cancer is mainly diagnosed clinically through colonoscopy, but its use for screening is limited. Detecting tumor markers specific for colon cancer is becoming increasingly important for its early diagnosis because the method is simple, convenient, and painless. Currently, CEA and CA19-9 are the main markers used for early screening of colorectal cancer. However, the sensitivity and accuracy of these markers are limited. Therefore, the search for sensitive and specific biomarkers is a hot topic of research.

MACC1 was first detected in colorectal cancer, and its expression in tissues is used as an independent marker for predicting metastasis and evaluating the prognosis of this malignancy [2]. As a key regulator of the HGF/Met signaling pathway, MACC1 is involved in tumor cell growth, cell migration and invasion, tumor angiogenesis, epithelial-mesenchymal transition, and other important pathophysiological processes. It can also form a positive feedback loop and further promote tumor cell invasion and metastasis. Our preliminary studies also showed that MACC1 is involved in the development of chemoresistance in pancreatic cancer patients by regulating the activation of the Ras/ERK signaling pathway [8]. In addition, follow-up studies also suggested that MACC1 plays an important role in signal transduction pathways, such as Akt/β-catenin, PI3K/AKT, and TWIST1/VEGF-A [11-15]. MACC1 was not only highly expressed in colorectal cancer but also in other malignant tumors; it was also closely related to the metastasis and prognosis of

Table 2. Univariate and multivariate Cox analysis of variables considered for overall survival rates of colorectal cancer patients

Variables	Univariate			Multivariate		
	HR	95% CI	*P* value	HR	95% CI	*P* value
Age	1.077	0.662-1.752	0.766			
Gender	1.139	0.668-1.994	0.632			
Lymph Node Metastasis	3.82	2.760-5.287	< 0.001	1.709	1.052-2.778	0.031
Tumor Deposit	5.378	3.274-8.835	< 0.001	0.976	0.482-1.976	0.946
Vascular Invasion	3.18	1.885-5.367	< 0.001	2.026	1.147-3.578	0.015
Nerve Invasion	6.28	3.814-10.343	< 0.001	1.565	0.771-3.178	0.215
Distant Metastasis	3.532	2.702-4.618	< 0.001	2.405	1.673-3.457	< 0.001
CEA Status	4.131	2.435-7.008	< 0.001	2.706	1.518-4.824	0.001
CA19-9 Status	4.142	2.533-6.775	< 0.001	0.833	0.429-1.618	0.59
MACC1 Status	2.8	1.524-5.144	0.001	1.279	0.651-2.512	0.475

those tumors [16-18]. Results of our preliminary meta-analysis also showed a statistically significant correlation between the high expression of MACC1 and short OS and recurrence-free survival in cancer patients (all $p < 0.001$) [10]. When MACC1 was discovered in 2009, Stein analyzed the differences of MACC1 levels in colon tumor tissue, metastatic foci, and healthy tissue and found that MACC1 expression was highest in the metastatic foci. By contrast, it was barely detected in normal colonic mucosa. They also assessed MACC1 expression at the gene level, and the results were consistent with the protein findings [2]. A recent study by Ashktorab. also showed that MACC1 expression in the colonic tissue was significantly higher in patients with colorectal cancer than that in healthy controls ($p = 0.014$) [19]. However, the serum expression of MACC1 in patients with colorectal cancer patients has not been reported.

In the present study, ELISA detection of serum MACC1 expression showed that serum MACC1 levels in colorectal cancer patients were significantly higher than that in healthy controls. This was the first time that the expression of serum MACC1 was studied and verified, and the results are consistent with those of Stein. By correlating MACC1 levels with the clinicopathological features of the patients, we further found that high MACC1 expression was associated with various clinical features of colon cancer, including lymph node metastasis, distant metastasis, neural invasion, tumor nodule status, and high TNM stage, among others. These data showed that high MACC1 expression was significantly related to malignant behavior and tumor progression of colorectal cancer. Survival analysis showed that abnormal serum MACC1 expression can be used as a biological indicator of poor prognosis in colorectal cancer patients. These results are in concordance with those of other studies. However, our study is unique in that we detected the serum MACC1 expression in colorectal cancer patients using ELISA, and this has high clinical value. Compared with the common methods for histological detection of tumor-specific indicators, serum samples are more convenient to obtain and easier to promote and apply in the clinic, meaning that this method has better feasibility for early screening and long-term follow-up of cancer patients. Concurrently, this study has some limitations because of its small sample size, short follow-up time, and the exclusion of patients who received postoperative adjuvant therapy. Therefore, further research with large sample sizes and active multicenter clinical studies are needed to validate our results.

In summary, abnormal MACC1 expression is significantly related to the biological behavior of colorectal cancer. Detecting the serum expression of MACC1 has important clinical value in the early diagnosis, malignancy prediction, and prognosis evaluation of colorectal cancer. MACC1 expression is an ideal evaluation index because of its convenient sampling, fast detection, and specificity. Further exploration of the molecular mechanisms and role of MACC1 in the progression of colorectal cancer would be beneficial in developing new proce-

dures for the diagnosis and treatment of colorectal cancer.

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Disclosure of conflict of interest

None.

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