Int J Clin Exp Pathol 2017;10(10):10441-10450
www.ijcep.com /ISSN:1936-2625/IJCEP0060088

Original Article

Meprin α combined with CEA and CA19-9 improves prognostic prediction for surgically treated colorectal cancer patients

Hongfa Hou[1*], Xinmin Gou[2*], Juyuan Bu[1], Yonghui Su[1], Xinying Wei[1], Xiao Wang[1], Bingzong Hou[1]

Departments of [1]Gastrointestinal Surgery, [2]Pathology, Fifth Affiliated Hospital of Sun Yat-sen University, Zhuhai, Guangdong Province, China. [*]Equal contributors.

Received June 22, 2017; Accepted August 16, 2017; Epub October 1, 2017; Published October 15, 2017

Abstract: Background: Carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) are generally used as tumour markers in patients with colorectal cancer (CRC), and meprin α might be an additional marker. Methods: The preoperative expression of serum CEA and CA19-9 was evaluated using a C12 protein biochip system, and tissue meprin α expression in CRC cells was detected by immunohistochemistry. The relationships of these indexes with clinicopathological parameters and the survival of CRC patients were analysed. Results: Of the 147 CRC patients, the preoperative seropositive rates for CEA and CA19-9 were 51.70% and 44.22%, respectively, and the tissue meprin α positive rate was 39.46%. Preoperative seropositivity for CEA was correlated with tumour size (P = 0.019), T stage (P = 0.005) and staging of CRC based on the American Joint Committee on Cancer (AJCC) guidelines (P = 0.032). The preoperative seropositive rate for CA19-9 was correlated with AJCC tumour stage (P = 0.031). High expression of meprin α was significantly correlated with distant CRC metastasis (P = 0.003), serum CEA (P = 0.002) and serum CA19-9 (P = 0.001). The combination of the three markers was an independent prognostic factor in patients with CRC (HR 3.985, 95% CI 1.106-14.361, P = 0.035 for overall survival). Conclusions: Tissue meprin α expression may be a useful predictor of metastasis and prognosis in CRC. The combined detection of the three markers may also be helpful to improve the accuracy of CRC prognosis monitoring.

Keywords: Colorectal cancer, meprin α, CEA, CA19-9, prognosis

Introduction

Colorectal cancer (CRC) is one of the three most prevalent malignancies and a leading cause of cancer-related death worldwide [1, 2]. Despite advances in surgical and adjuvant systemic regimens, the long-term survival of individuals with CRC is unsatisfactory due to tumour recurrence and metastasis. When CRC is detected at a localized stage (39% of cases), the 5-year relative survival rate is 90%, but once distant metastasis occurs, the 5-year survival is only 20% [1].

Five decades after Gold and Freedman first described extracting tumour-associated carcinoembryonic antigen (CEA) from human CRC tissues [3], CEA is now a widely recognized member of the immunoglobulin superfamily that plays a unique role in intracellular adhesion [4]. CEA is expressed in malignant tissues, and particularly gastrointestinal carcinomas, as well as in benign disease and healthy individuals. To date, the evidence-based clinical practice guidelines of American Society of Clinical Oncology (ASCO) and the European Group on Tumor Markers (EGTM) recommend that CEA be used in predicting prognosis, in surveillance following radical operation and in monitoring therapeutic effects in advanced CRC [5, 6]. Serum CEA is elevated in 38.5-81.0% [7-9] of CRC patients, so poor stability has limited its performance in clinical application.

Carbohydrate antigen 19-9 (CA19-9) was originally conceived as a CRC marker but has been used clinically as a pancreatic cancer marker [5] and is a ligand for E-selectin that plays an important role in the adhesion of cancer cells to endothelial cells [10]. CA19-9 levels are eleva-

ted in approximately 14.16-66.69% of CRC patients [7, 9, 11]. Generally, most studies have supported the efficacy of CA19-9 as an adverse prognostic factor for CRC patients. However, in contrast to CEA, CA19-9 is now recognized as a less sensitive marker in CRC and is often used in combination with CEA to manage CRC patients [5, 6]. However, the precise correlation between CA19-9 and CRC prognosis requires further study.

Meprin (EC 3.4.24.18) belongs to the astacin family of zinc endopeptidases and the metzincin superfamily and is mainly encoded by MEP1A on chromosome 6. Meprin is expressed in numerous tissues, organs and cell types and exhibits potent activity in hydrolysing a variety of peptide and protein substrates [12], such as hormones, growth factors, extracellular matrix (ECM) proteins, and secreted protein kinases, resulting in activation or inactivation of these molecules [13]. As a result, meprin has functions in angiogenesis, cancer, inflammation, fibrosis and neurodegenerative diseases [14, 15]. There are two homologous isoforms of meprin: meprin α, encoded by MEP1A on chromosome 6, and meprin β, encoded by MEP1B on chromosome 18 [16, 17].

Abnormal meprin α expression has been studied in some detail in the intestine, skin, and kidney and also in leukocytes and several types of cancer [14]. Meprin α is expressed both in epithelial cells of the healthy colonic mucosa and in CRC [18]. Our previous research demonstrated that increased meprin α levels are seen in CRC and that CRC cellular proliferation and invasiveness are inhibited when meprin α expression is dampened [19]. A similar investigation was conducted by Daniel Lottaz and colleagues [20]. To the best of our knowledge, so far, the significance of the relationships among the preoperative serum levels of CEA, CA19-9 and tissue meprin α in CRC has not been reported.

In the current study, we aimed to further assess the significance of the preoperative levels of the serum tumour markers CEA, CA19-9, and tissue meprin α and the utility of combining the three markers in predicting the prognosis of patients undergoing surgery for CRC. The relationships among the levels of the preoperative serum tumour markers CEA, CA19-9 and tissue meprin α were also explored.

Materials and methods

Patient selection

Information on all patients who underwent surgery for CRC in the Department of General Surgery, Fifth Affiliated Hospital of Sun Yat-sen University, between November 2008 and December 2011 was collected in a database. All patients underwent surgery by the same surgical team and provided informed consent in advance. The enrolment criteria were as follows: (i) not having other malignancy, hereditary colon cancer, and inflammatory bowel disease and having received any anticancer treatment prior to surgery, (ii) having undergone radical excision, (iii) having a CRC diagnosis confirmed by postoperative histopathology, (iv) having undergone pre-surgical serum CEA and CA19-9 concentration testing, and (v) having available follow-up information. The clinicopathological features and staging were determined according to the American Joint Committee on Cancer (AJCC) TNM classification guidelines [21]. The study was undertaken with approval by the Institutional Review Board of the Fifth Affiliated Hospital of Sun Yat-sen University.

Serum CEA and CA19-9 level quantitation

Preoperative peripheral blood was collected in 5 ml serum storage tubes and centrifuged at 3,000×g for 10 min at room temperature, followed by storage at -80°C until use. The serum CEA and CA19-9 levels were routinely measured using a C12 protein biochip system (Shanghai Health-Digit Co, Ltd, China) [22]. The cutoff values for CEA and CA19-9 were 5 ng/ml and 35 U/ml, respectively, according to the manufacturer's instructions, and a value below the cutoff was considered negative.

Immunohistochemical examinations

In each case, a representative histological specimen at the deepest area invaded by CRC was selected for immunohistochemistry (IHC). Sections (thickness, 4 μm) were mounted on silane-coated glass slides (Fuzhou Maixin Biotech. Co., Ltd.), dewaxed in xylene, and rehydrated in a graded series of ethanol. For thermal remediation of the antigen meprin α, each section was heated in citrate-buffered solution (pH 6.0) at 100°C for 20 min. The sections were then immersed in a 0.3% hydrogen perox-

ide solution for 10 min at room temperature to block endogenous peroxidase activity and rinsed with phosphate-buffered saline (PBS) three times for 3 min each. Thereafter, 50 μL of meprin α-specific primary antibody working solution (at a dilution of 1:200; R&D Systems Inc., USA) was added to every section, and the sections were incubated at 4°C overnight and then washed with PBS again three times for 3 min each. Subsequently, staining was performed with HRP-conjugated secondary antibody (Dako Cytomation, Glostrup, Denmark) with incubation for 15 min, again followed by washes with PBS. Finally, the sections were reacted with 3,3'-diaminobenzidine tetrahydrochloride (DAB), dehydrated in a graded ethanol series, and mounted with coverslips. All the above procedures were performed according to the manufacturers' instructions.

Immunohistochemical evaluation

Two trained investigators who were blinded to the clinicopathological data examined the slides under a light microscope (BX43 microscope; Olympus, Tokyo, Japan) and scored the slides independently. Each slide was scored based on the intensity and the percentage of positively stained malignant cytoplasm, and the mean values for four representative fields were determined at a magnification of ×200. The score for each section was measured as A×B, as described previously [19, 23]. Immunostaining intensity (A) was scored as 0 (lack of staining), 1 (weak staining), 2 (moderate staining), or 3 (Strong staining), and the proportion of positively stained tumour cells (B) was scored as 0 (< 5%), 1 (6-25%), 2 (26-50%), 3 (51-75%), or 4 (> 75%). Case with protein IHC scores above 3 were defined as being positive.

Follow-up

Survival data were obtained from hospital medical charts and by contacting patients or their families through telephone calls following a standardized protocol. Patients were followed up at 3-month intervals for the first 2 years and then at 6-month intervals thereafter. Overall survival (OS) was calculated from the surgical date to death from CRC or the date of last call (December 30, 2016), whichever occurred first. The median follow-up time was 51 months (range, 26-96 months).

Statistical analysis

SPSS version 20.0 software for Windows (IBM, Armonk, NY, USA) was used for statistical analysis. Pearson's chi-square test or Fisher's exact test was used to analyse the relationship between serum antigen and CRC clinicopathological features. Correlations among CEA, CA19-9 and meprin α were determined using Pearson's correlation analysis. The Kaplan-Meier method was used to plot survival curves, which were compared using the log-rank test. Univariate and multivariate analyses of OS were performed using the Cox proportional hazard model. The confidence intervals (CIs) were set to 95%, and $P < 0.05$ (two-sided) was considered statistically significant.

Results

Demographic data

In strict accordance with our enrolment criteria, 147 patients were ultimately enrolled in our retrospective study. The patients had a median age of 64 years (range, 25-94 years); 77 were males (52.38%), and 70 were females (47.62%). At surgery, 66 (44.90%) cases had positive lymph nodes, whereas 81 (55.10%) cases were negative, and 36 (24.49%) had distant metastasis. There were 74 (54.4%) cases of stage I or II tumours and 62 (45.6%) cases of stage III or IV tumours based on the AJCC TNM classification criteria [21]. In total, 38 (25.85%) tumours were poorly differentiated adenocarcinomas, and 109 (74.15%) were well or moderately differentiated adenocarcinomas (**Table 1**).

Characteristics of patients relative to tumour markers

The median preoperative CEA and CA19-9 concentrations were 5.53 ng/ml (mean, 33.25; range, 0.20-703.28) and 27.03 U/ml (mean, 61.19; range, 0.60-555.22), respectively. Of the 147 patients, 76 (51.70%) manifested preoperative CEA seropositivity (≥ 5 ng/ml), and 65 (44.22%) presented preoperative CA19-9 seropositivity (≥ 35 U/ml). The positive rates for CEA and CA19-9 were 51.70% and 44.22%, respectively. Preoperative CEA seropositivity significantly correlated with tumour size ($P = 0.019$), T stage ($P = 0.005$) and AJCC stage ($P = 0.032$). Preoperative CA19-9 seropositivity was associated with AJCC tumour stage ($P = 0.023$) (**Table 1**).

Table 1. Characteristics of 147 CRC patients

Variable	n	CEA- (n = 71)	CEA+ (n = 76)	*P*-value	CA19-9- (n = 82)	CA19-9+ (n = 65)	*P*-value	Meprin α- (n = 89)	Meprin α+ (n = 58)	*P*-value
Age (years)										
< 65	74	37	37	0.742	42	32	0.869	46	28	0.737
≥ 65	73	34	39		40	33		43	30	
Gender										
Female	70	29	41	0.137	41	29	0.618	43	27	0.867
Male	77	42	35		41	36		46	31	
Location										
Colon	104	54	50	0.206	62	42	0.201	65	39	0.464
Rectum	43	17	26		20	23		24	19	
Size (cm)										
< 5	86	49	37	0.019*	46	40	0.613	55	31	0.392
≥ 5	61	22	39		36	25		34	27	
T stage										
T1+T2	49	32	17	0.005*	29	20	0.600	33	16	0.284
T3+T4	98	39	59		53	45		56	42	
N stage										
N0	81	44	37	0.135	49	32	0.243	52	29	0.396
N1+N2	66	27	39		33	33		37	29	
M stage										
M0	111	58	53	0.124	65	46	0.252	75	36	0.003*
M1	36	13	23		17	19		14	22	
AJCC stage										
I+II	67	39	28	0.032*	44	23	0.031*	46	21	0.090
III+IV	80	32	48		38	42		43	37	
Differentiation										
Well + Moderate	109	54	55	0.707	62	47	0.706	71	38	0.082
Poor	38	17	21		20	18		18	20	

*$P < 0.05$.

Meprin α is predominantly expressed on the cell membrane of tumour cells. Among the 147 CRC tissue samples examined in the current study, 58 (39.46%) exhibited positive immunostaining for the meprin α protein, and 89 (60.54%) exhibited negative immunostaining. High expression of meprin α in CRC tissues was significantly correlated with CRC metastasis ($P = 0.003$) (**Table 1**). Representative IHC images for meprin α staining can be seen in **Figure 1**.

Correlation between tumour markers

Pearson's correlation analysis demonstrated that CEA was positively correlated with meprin α ($P = 0.002$, r = 0.251), but not with CA19-9 ($P = 0.146$, r = 0.120), whereas CA19-9 was positively correlated with meprin α ($P = 0.001$, r = 0.262). Additional statistics are shown in **Table 2**.

Combined detection of CEA, CA19-9 and meprin α

The combined effect was rated by considering the combined value as negative as long as expression of all three markers was negative; otherwise, the combined value was evaluated as positive. The combination of the three biomarkers increased the positive rate from 51.70% to 70.07%.

Relationships between tumour markers and patient outcomes

The expression of CEA, CA19-9 and meprin α was analysed using Kaplan-Meier survival

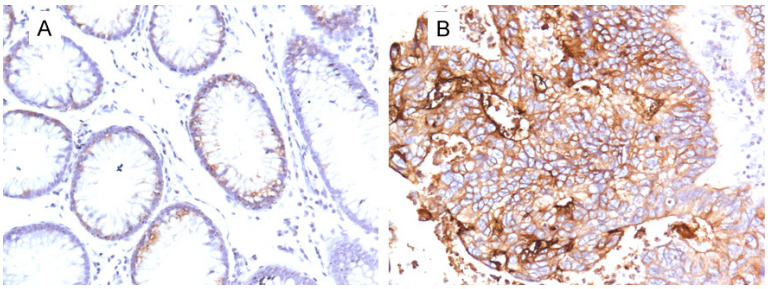

Figure 1. Immunohistochemical analysis of meprin α protein (magnification, ×200). A. Meprin α is rarely expressed in the normal mucosa; B. Meprin α is intensely expressed in the cell membrane of tumour cells.

Table 2. Correlation among the tumour markers CEA, CA19-9 and meprin α

		CEA	CA19-9	Meprin α
CEA	Pearson's correlation	1	0.120	0.251
	Significance (two-tailed)		0.146	0.002**
	N	147	147	147
CA19-9	Pearson's correlation	0.120	1	0.262
	Significance (two-tailed)	0.146		0.001**
	N	147	147	147
Meprin α	Pearson's correlation	0.251	0.262	1
	Significance (two-tailed)	0.002**	0.001**	
	N	147	147	147

**Significant at level of $P = 0.01$ (two-tailed).

curves. The significance in terms of OS was assessed using the log-rank test. Compared with low concentrations, augmented preoperative serum CEA and CA19-9 concentrations, high expression of meprin α in CRC tissues and the combination of the three markers appeared to be associated with shorter OS ($P = 0.035$, 0.019, 0.013 and 0.005, respectively) (**Figure 2A-D**).

Prognostic factors for CRC

According to the univariate analysis, age, tumour size, T stage, N stage, M stage, AJCC stage, histopathological grading, CEA level, CA19-9 level, meprin α level and the combination of the three markers showed noticeable correlations with poor OS ($P = 0.041$, $P = 0.046$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P = 0.038$, $P = 0.021$, $P = 0.015$, and $P = 0.007$, respectively) (**Table 3**). The multivariate analysis demonstrated that T stage, M stage, AJCC stage, histopathological grading and the combination of the three markers were independent predictive factors for survival in patients with CRC (HR 3.432, 95% CI 1.282-9.187, $P = 0.014$; HR 2.825, 95% CI 1.394-5.725, $P = 0.004$; HR 4.061, 95% CI 1.268-13.011, $P = 0.018$; HR 2.150, 95% CI 1.256-3.680, $P = 0.005$; and HR 3.985, 95% CI 1.106-14.361, $P = 0.035$, respectively) (**Table 3**).

Discussion

Our presented data support a significant relationship between aberrant expression of meprin α in CRC tissues and CRC metastasis. The underlying molecular mechanism might involve proteolytic activation and/or the release of pro-angiogenic growth factors, such as vascular endothelial growth factor A (VEGF-A) and connective tissue growth factor (CTGF), which have both been identified as meprin substrates [24, 25]. In cultured colon carcinoma cells (Caco-2), meprin α is secreted both apically and basolaterally, resulting in an abnormal accumulation of meprin α activity in the tumour stroma [26]. Consequently, the capacity of meprin α is directed towards the ECM, where it can facilitate the destruction of stromal structure, thereby affecting the proliferation and invasiveness of tumour cells into the surrounding tissue. Our previous research demonstrated that both in vitro and in vivo, CRC cellular proliferation and migration were inhibited when MEP1A expression was inhibited [19]. In addition, Daniel Lottaz and his colleagues showed that meprin α directly promotes cell migration, although the pro-angiogenic effect of meprin α might be even more critical for cancer pathology [20]. Additionally, the cell-culture experiments conducted by Gail L. Matters and her colleagues demonstrated that human breast carcinoma cells treated with a meprin α inhibitor (actinonin) resulted in decreased invasiveness in vitro [27]. The comprehensive and specific cytological and molecular biological mechanisms remain to be further investigated.

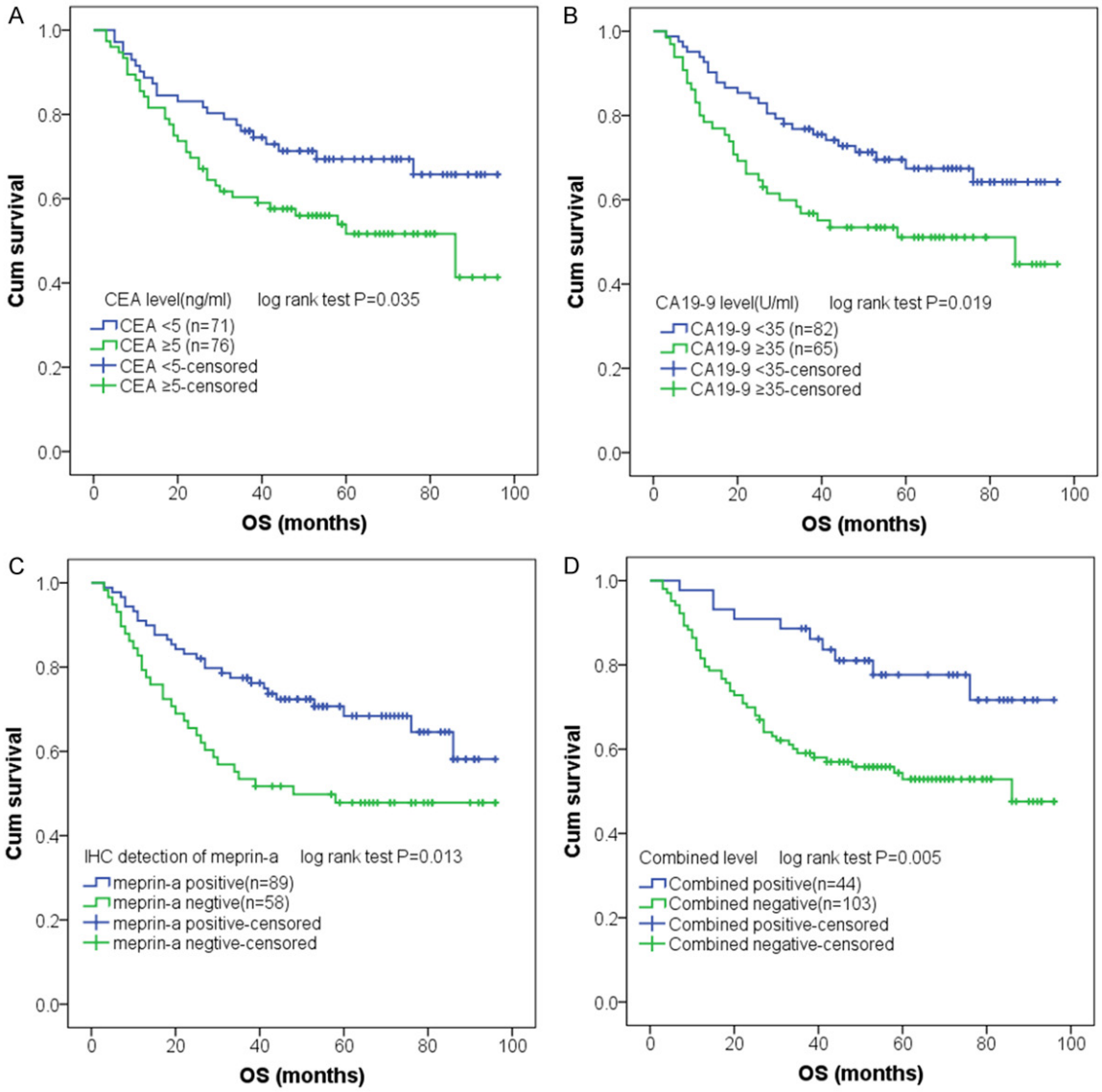

Figure 2. Kaplan-Meier survival analysis of patients with CRC. A. Preoperative serum CEA levels and OS; B. Preoperative serum CA19-9 levels and OS; C. Expression level of Meprin α in tumour cells and OS; D. Combined detection of CEA, CA19-9 and meprin α expression levels and OS.

A series of studies have indicated that CEA is involved in multiple biological aspects of neoplasia, such as cell adhesion, metastasis, suppression of cellular immune mechanisms, and inhibition of apoptosis [28-31]. CEA is involved in cell-to-cell adhesion and has a dominant effect in blocking cell differentiation [32], and it coordinates with Myc and Bcl-2 in cellular transformation [33]. In addition, CEA overexpression directly attenuates TGF-β signalling, regulating both tumour apoptosis and metastasis [34]. For instance, CEA boosts weakly metastatic CRC to colonize the liver and develop spontaneous haematogeneous liver and lung metastases [35]. It has been extensively documented that CEA expression also correlates well with resistance to anoikis [36] and with cell differentiation [33]. However, it is still controversial whether CEA is an independent prognostic factor in metastatic CRC [37-39]. Our results allow us to conclude that preoperative serum CEA is a prognostic factor in CRC but has no role in the diagnosis of CRC metastasis and is not an independent prognostic factor.

CA19-9 was originally identified as a colon cancer antigen but has been used clinically as a pancreatic cancer marker [5], and it is a ligand

Table 3. Univariate and multivariate analyses of OS

Variable	Univariate	*P*-value	Multivariate	*P*-value
	HR (95% CI)		HR (95% CI)	
Age (years)				
< 65	1	0.041*	1	0.170
≥ 65	1.730 (1.022-2.928)		1.464 (0.850-2.524)	
Gender				
Female	1	0.620		
Male	0.878 (0.524-1.470)			
Location				
Colon	1	0.152		
Rectum	1.480 (0.866-2.529)			
Size (cm)				
< 5	1	0.046*	1	0.986
≥ 5	1.691 (1.010-2.831)		0.995 (0.576-1.719)	
T stage				
T1+T2	1	< 0.001*	1	0.014*
T3+T4	7.499 (2.991-18.802)		3.432 (1.282-9.187)	
N stage				
N0	1	< 0.001*	1	0.920
N1+N2	3.281 (1.892-5.690)		0.961 (0.447-2.067)	
M stage				
M0	1	< 0.001*	1	0.004*
M1	6.312 (3.698-10.774)		2.825 (1.394-5.725)	
AJCC stage				
I+II	1	< 0.001*	1	0.018*
III+IV	8.102 (3.825-17.163)		4.061 (1.268-13.011)	
Differentiation				
Well + Moderate	1	< 0.001*	1	
Poor	2.780 (1.649-4.687)		2.150 (1.256-3.680)	0.005*
CEA (Negative vs. Positive)	1.754 (1.031-2.985)	0.038*	0.581 (0.270-1.250)	0.165
CA19-9 (Negative vs. Positive)	1.840 (1.096-3.089)	0.021*	0.996 (0.484-2.050)	0.991
Meprin α (Negative vs. Positive)	1.899 (1.134-3.182)	0.015*	0.767 (0.384-1.531)	0.453
Combined 3 markers (Negative vs. Positive)	2.541 (1.285-5.027)	0.007*	3.985 (1.106-14.361)	0.035*

*$P < 0.05$.

for E-selectin that has an important effect on cancer invasion by enhancing cell adhesion to endothelial cells and promoting angiogenesis indirectly [10, 40]. In our study, we observed that preoperative CA19-9 seropositivity was significantly associated with shorter OS, consistent with published research, but the multivariate Cox proportional hazard model confirmed that high CA19-9 expression was not an independent prognostic factor.

In our research, we observed a significantly enhanced serum CEA concentration in CRC patients with a tumour size larger than 5 cm, which may be ascribed to the fact that larger tumours possess more tumour cells, suggesting a potential correlation between the CEA concentration and tumour mass. Contrary to certain studies [9, 41], our data indicated that none of the three biomarkers varies significantly among the different stages of histological differentiation of CRC. Multicentre investigations with larger sample sizes are needed to settle this dispute.

The present research confirmed that preoperative CEA and CA19-9 seropositivities were associated with aggressive CRC according to T

and AJCC stages. The conclusions are broadly in line with published research [8, 42, 43]. We also discovered that preoperative CEA, CA19-9 seropositivity and aberrant tissue meprin α expression were associated with advanced disease and adverse outcomes. Interestingly, although preoperative CEA, CA19-9 seropositivity and aberrant tissue meprin α expression in CRC were associated with poor prognosis, none of them was an independent prognostic biomarker according to our current research. Multivariate analysis suggested that the combined detection of preoperative serum CEA, CA19-9 and meprin α expression in tumour cells was an adverse independent factor for the survival of CRC patients ($P = 0.035$). These data implied that combined detection of the expression of the three markers in CRC tissues is helpful for grading and postoperative monitoring of CRC.

Our study has several limitations. First, it had a retrospective and single-centre design with a relatively small number of patients. Thus, the analysis of the relationship between biomarkers and disease outcomes may have been affected by subjective bias. Second, we were unable to analyse the possible relationship between tumour markers and disease-free survival (DFS) due to a lack of adequate follow-up data. Finally, we also failed to separately analyse the outcomes of CRC according to different postoperative treatments, such as chemotherapy, radiotherapy and other adjuvant therapies, which should be the focus of future studies.

In conclusion, preoperative CEA, CA19-9 seropositivity and tissue meprin α expression were significantly associated with a poor prognosis in patients with CRC. Nevertheless, none of them seems to be an independent prognostic factor. Age; tumour size; TNM stage; AJCC stage; differentiation; and combined detection of CEA, CA19-9 and meprin α were also related to poor outcome. In addition, T stage; M stage; AJCC stage; differentiation; and combined detection of CEA, CA19-9 and meprin α were independent prognostic factors for CRC. A combined analysis of these three markers provided sufficient data for clinical assessment and management of CRC, in contrast to analysis of solely a single tumour marker. High expression of meprin α was a good indicator of CRC metastasis, and meprin α suppression might be a therapeutic strategy for inhibiting CRC metastasis. Multicentre, prospective studies on the predictive role of serum tumour markers should be conducted to verify these results.

Acknowledgements

This work was funded by the Zhuhai City, Guangdong Province Science and Technology Department (No.20171009E030027).

Disclosure of conflict of interest

None.

Address correspondence to: Drs. Bingzong Hou and Xiao Wang, Department of Gastrointestinal Surgery, Fifth Affiliated Hospital of Sun Yat-sen University, Zhuhai 519000, Guangdong Province, China. Tel: +86-756-2528708; Fax: +86-0756-63241377; E-mail: sysuhoubz@163.com (BZH); hnwangx@163.com (XW)

References

[1] Siegel RL, Miller KD and Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016; 66: 7-30.

[2] Van Cutsem E, Cervantes A, Nordlinger B and Arnold D. Metastatic colorectal cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol 2014; 25 Suppl 3: iii1-9.

[3] Gold P and Freedman SO. Specific carcinoembryonic antigens of the human digestive system. J Exp Med 1965; 122: 467-481.

[4] Hammarstrom S. The carcinoembryonic antigen (CEA) family: structures, suggested functions and expression in normal and malignant tissues. Semin Cancer Biol 1999; 9: 67-81.

[5] Locker GY, Hamilton S, Harris J, Jessup JM, Kemeny N, Macdonald JS, Somerfield MR, Hayes DF and Bast RC Jr. ASCO 2006 update of recommendations for the use of tumor markers in gastrointestinal cancer. J Clin Oncol 2006; 24: 5313-5327.

[6] Duffy MJ, Lamerz R, Haglund C, Nicolini A, Kalousova M, Holubec L and Sturgeon C. Tumor markers in colorectal cancer, gastric cancer and gastrointestinal stromal cancers: European group on tumor markers 2014 guidelines update. Int J Cancer 2014; 134: 2513-2522.

[7] Kadam PD and Chuan HH. Erratum to: rectocutaneous fistula with transmigration of the suture: a rare delayed complication of vault fixation with the sacrospinous ligament. Int Urogynecol J 2016; 27: 505.

[8] Park IJ, Choi GS, Lim KH, Kang BM and Jun SH. Serum carcinoembryonic antigen monitoring after curative resection for colorectal cancer:

clinical significance of the preoperative level. Ann Surg Oncol 2009; 16: 3087-3093.
[9] Zhong W, Yu Z, Zhan J, Yu T, Lin Y, Xia ZS, Yuan YH and Chen QK. Association of serum levels of CEA, CA199, CA125, CYFRA21-1 and CA72-4 and disease characteristics in colorectal cancer. Pathol Oncol Res 2015; 21: 83-95.
[10] Sato H, Usuda N, Kuroda M, Hashimoto S, Maruta M and Maeda K. Significance of serum concentrations of E-selectin and CA19-9 in the prognosis of colorectal cancer. Jpn J Clin Oncol 2010; 40: 1073-1080.
[11] Park IJ, Choi GS and Jun SH. Prognostic value of serum tumor antigen CA19-9 after curative resection of colorectal cancer. Anticancer Res 2009; 29: 4303-4308.
[12] Bond JS and Beynon RJ. The astacin family of metalloendopeptidases. Protein Sci 1995; 4: 1247-1261.
[13] Kruse MN, Becker C, Lottaz D, Kohler D, Yiallouros I, Krell HW, Sterchi EE and Stocker W. Human meprin alpha and beta homo-oligomers: cleavage of basement membrane proteins and sensitivity to metalloprotease inhibitors. Biochem J 2004; 378: 383-389.
[14] Broder C and Becker-Pauly C. The metalloproteases meprin alpha and meprin beta: unique enzymes in inflammation, neurodegeneration, cancer and fibrosis. Biochem J 2013; 450: 253-264.
[15] Bond JS, Matters GL, Banerjee S and Dusheck RE. Meprin metalloprotease expression and regulation in kidney, intestine, urinary tract infections and cancer. FEBS Lett 2005; 579: 3317-3322.
[16] Bond JS, Rojas K, Overhauser J, Zoghbi HY and Jiang W. The structural genes, MEP1A and MEP1B, for the alpha and beta subunits of the metalloendopeptidase meprin map to human chromosomes 6p and 18q, respectively. Genomics 1995; 25: 300-303.
[17] Bertenshaw GP, Norcum MT and Bond JS. Structure of homo- and hetero-oligomeric meprin metalloproteases. Dimers, tetramers, and high molecular mass multimers. J Biol Chem 2003; 278: 2522-2532.
[18] Lottaz D, Hahn D, Muller S, Muller C and Sterchi EE. Secretion of human meprin from intestinal epithelial cells depends on differential expression of the alpha and beta subunits. Eur J Biochem 1999; 259: 496-504.
[19] Wang X, Chen J, Wang J, Yu F, Zhao S, Zhang Y, Tang H and Peng Z. Metalloproteases meprin-α (MEP1A) is a prognostic biomarker and promotes proliferation and invasion of colorectal cancer. BMC Cancer 2016; 16: 383.
[20] Lottaz D, Maurer CA, Noel A, Blacher S, Huguenin M, Nievergelt A, Niggli V, Kern A, Muller S, Seibold F, Friess H, Becker-Pauly C, Stocker W and Sterchi EE. Enhanced activity of meprin-alpha, a pro-migratory and pro-angiogenic protease, in colorectal cancer. PLoS One 2011; 6: e26450.
[21] Chen VW, Hsieh MC, Charlton ME, Ruiz BA, Karlitz J, Altekruse SF, Ries LA and Jessup JM. Analysis of stage and clinical/prognostic factors for colon and rectal cancer from SEER registries: AJCC and collaborative stage data collection system. Cancer 2014; 120: 3793-3806.
[22] Chen C, Chen LQ, Yang GL and Li Y. The application of C12 biochip in the diagnosis and monitoring of colorectal cancer: systematic evaluation and suggestion for improvement. J Postgrad Med 2008; 54: 186-190.
[23] Ma YL, Peng JY, Zhang P, Liu WJ, Huang L and Qin HL. Immunohistochemical analysis revealed CD34 and Ki67 protein expression as significant prognostic factors in colorectal cancer. Med Oncol 2010; 27: 304-309.
[24] Jefferson T, dem Keller UA, Bellac C, Metz VV, Broder C, Hedrich J, Ohler A, Maier W, Magdolen V, Sterchi E, Bond JS, Jayakumar A, Traupe H, Chalaris A, Rose-John S, Pietrzik CU, Postina R, Overall CM and Becker-Pauly C. The substrate degradome of meprin metalloproteases reveals an unexpected proteolytic link between meprin beta and ADAM10. Cell Mol Life Sci 2013; 70: 309-333.
[25] Schutte A, Hedrich J, Stocker W and Becker-Pauly C. Let it flow: morpholino knockdown in zebrafish embryos reveals a pro-angiogenic effect of the metalloprotease meprin alpha2. PLoS One 2010; 5: e8835.
[26] Lottaz D, Maurer CA, Hahn D, Buchler MW and Sterchi EE. Nonpolarized secretion of human meprin alpha in colorectal cancer generates an increased proteolytic potential in the stroma. Cancer Res 1999; 59: 1127-1133.
[27] Matters GL, Manni A and Bond JS. Inhibitors of polyamine biosynthesis decrease the expression of the metalloproteases meprin alpha and MMP-7 in hormone-independent human breast cancer cells. Clin Exp Metastasis 2005; 22: 331-339.
[28] Kim KS, Kim JT, Lee SJ, Kang MA, Choe IS, Kang YH, Kim SY, Yeom YI, Lee YH, Kim JH, Kim KH, Kim CN, Kim JW, Nam MS and Lee HG. Overexpression and clinical significance of carcinoembryonic antigen-related cell adhesion molecule 6 in colorectal cancer. Clin Chim Acta 2013; 415: 12-19.
[29] Grimm T, Riethmuller G and Johnson JP. Characteristics of carcinoembryonic antigen (CEA) expressed in different cell types: evidence that CEA can function as an adhesion molecule and as a repulsion molecule. Biochem Biophys Res Commun 1994; 204: 1225-1234.

[30] Ordonez C, Screaton RA, Ilantzis C and Stanners CP. Human carcinoembryonic antigen functions as a general inhibitor of anoikis. Cancer Res 2000; 60: 3419-3424.
[31] Screaton RA, DeMarte L, Draber P and Stanners CP. The specificity for the differentiation blocking activity of carcinoembryonic antigen resides in its glycophosphatidyl-inositol anchor. J Cell Biol 2000; 150: 613-626.
[32] Benchimol S, Fuks A, Jothy S, Beauchemin N, Shirota K and Stanners CP. Carcinoembryonic antigen, a human tumor marker, functions as an intercellular adhesion molecule. Cell 1989; 57: 327-334.
[33] Screaton RA, Penn LZ and Stanners CP. Carcinoembryonic antigen, a human tumor marker, cooperates with Myc and Bcl-2 in cellular transformation. J Cell Biol 1997; 137: 939-952.
[34] Li Y, Cao H, Jiao Z, Pakala SB, Sirigiri DN, Li W, Kumar R and Mishra L. Carcinoembryonic antigen interacts with TGF-{beta} receptor and inhibits TGF-{beta} signaling in colorectal cancers. Cancer Res 2010; 70: 8159-8168.
[35] Blumenthal RD, Osorio L, Hayes MK, Horak ID, Hansen HJ and Goldenberg DM. Carcinoembryonic antigen antibody inhibits lung metastasis and augments chemotherapy in a human colonic carcinoma xenograft. Cancer Immunol Immunother 2005; 54: 315-327.
[36] Samara RN, Laguinge LM and Jessup JM. Carcinoembryonic antigen inhibits anoikis in colorectal carcinoma cells by interfering with TRAIL-R2 (DR5) signaling. Cancer Res 2007; 67: 4774-4782.
[37] Wang WS, Lin JK, Chiou TJ, Liu JH, Fan FS, Yen CC, Lin TC, Jiang JK, Yang SH, Wang HS and Chen PM. CA19-9 as the most significant prognostic indicator of metastatic colorectal cancer. Hepatogastroenterology 2002; 49: 160-164.
[38] Aggarwal C, Meropol NJ, Punt CJ, Iannotti N, Saidman BH, Sabbath KD, Gabrail NY, Picus J, Morse MA, Mitchell E, Miller MC and Cohen SJ. Relationship among circulating tumor cells, CEA and overall survival in patients with metastatic colorectal cancer. Ann Oncol 2013; 24: 420-428.
[39] Carriquiry LA and Pineyro A. Should carcinoembryonic antigen be used in the management of patients with colorectal cancer? Dis Colon Rectum 1999; 42: 921-929.
[40] Ballehaninna UK and Chamberlain RS. The clinical utility of serum CA 19-9 in the diagnosis, prognosis and management of pancreatic adenocarcinoma: an evidence based appraisal. J Gastrointest Oncol 2012; 3: 105-119.
[41] Wada N, Kurokawa Y, Miyazaki Y, Makino T, Takahashi T, Yamasaki M, Nakajima K, Takiguchi S, Mori M and Doki Y. The characteristics of the serum carcinoembryonic antigen and carbohydrate antigen 19-9 levels in gastric cancer cases. Surg Today 2017; 47: 227-232.
[42] Thomas WM, Robertson JF, Price MR and Hardcastle JD. Failure of CA19-9 to detect asymptomatic colorectal carcinoma. Br J Cancer 1991; 63: 975-976.
[43] Lin PC, Lin JK, Lin CC, Wang HS, Yang SH, Jiang JK, Lan YT, Lin TC, Li AF, Chen WS and Chang SC. Carbohydrate antigen 19-9 is a valuable prognostic factor in colorectal cancer patients with normal levels of carcinoembryonic antigen and may help predict lung metastasis. Int J Colorectal Dis 2012; 27: 1333-1338.