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Original Article

Correlation analysis of the expression of mesenchymal circulating tumor cells and CD133 with the prognosis of colorectal cancer

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Abstract: Objective: To evaluate the expression of tumor stem cell marker CD133 in peripheral blood circulating tumor cells (CTC) and the value of CD133 in prognosis of patients with colorectal cancer (CRC). Methods: Preoperative/pre-chemotherapy peripheral blood samples of 63 patients with CRC from January 2016 to January 2021 were selected to detect peripheral blood CTC by CanPatrol CTC enrichment technology. Expression of CD133 in different epithelial-mesenchymal transition (EMT) typing CTC was analyzed. Clinical data (including tumor size, tumor stage, pathological typing, molecular typing, lymph node metastasis, distant metastasis, carcinoembryonic antigen (CEA), and CA-199 expression), PFS time and OS time were followed up. The expression of CD133 in different CTCs was compared, and the correlation between CD133 and patient survival time was compared. Results: The positive rate of E-CTC in patients with tumor diameter ≥5 cm was significantly higher than that of patients with tumor diameter <5 cm (P=0.035). The positive rate of M-CTC in patients with diabetes was significantly higher than that of patients without diabetes (P=0.006). The CD133-positive M-CTCs were significantly higher in DM and CEA>5 ng/mL patients than in non-DM and CEA≤5 ng/mL patients (P<0.001, P=0.0195). Fifty-five patients were followed up for a median of 14 months. During follow-up, 19 had disease progression and five died. According to the cutoff point obtained by ROC analysis, the PFS of M-CTC>2.5/5 ml patients (0%) was lower than that of ≤2.5/5 ml patients (76.5%), P<0.05. PFS in patients with CD133-positive M-CTC>0.5/5 mL (18.6%) was lower than in patients with ≤0.5/5 ml (76.5%), P<0.05. However, thedifference in the OS between patients with CD133-positive M-CTC>0.5/5 ml (71.7%) and those with ≤0.5/5 ml (93.8%), was not significant, P=0.054. Conclusion: CD133 positive M-CTC is closely related to distant metastasis in CRC. The expression of CD133 in CTC, especially in M-CTC, can be used as a prognostic indicator for colorectal cancer.

Keywords: Circulating tumor cells, epithelial-mesenchymal transition, CD133, colorectal cancer, prognosis

Introduction

Colorectal cancer (CRC) is the second leading cause of cancer-related deaths [1]. Most CRC patients die from advanced disease or relapse, mainly because of tumor metastasis [2]. CRC metastasis is a complex process made up of several steps involving multiple factors, and there is a need to better understand the drivers and mechanisms of CRC metastasis in order to develop strategies for better treatment or improved prognosis. The diagnosis and prognosis monitoring of CRC depend largely on tissue biopsy. However, liquid biopsies are a problem in CRC due to limitations such as the heterogeneous and aggressive nature of the tumor, high cost and limited applicability in therapeutic monitoring. Liquid biopsy is a non-invasive examination that can provide important diagnostic and prognostic information in many types of cancer [3]. Circulating tumor cells (CTC) in liquid biopsy are precursors of metastasis and show promise in the detection and diagnosis of primary tumors [4]. Existing studies have proven that CTC are closely related to a variety of clinicopathologic factors, that can predict a high risk of metastasis of different solid cancers (including breast cancer, hepatocellular carcinoma and ovarian cancer) [5-7]. In addition, CTCs are characterized by epithelial-mes-

Table 1. Epithelial Circulating Tumor Cell (E-CTC) distribution and baseline characteristics of patients [n (%)]

	E-CTC		P
	≥1 (n=30)	<1 (n=33)	
Age (years)			
≥60	20 (66.67)	15 (45.45)	0.091
<60	10 (33.33)	18 (54.55)	
Gender			
Male	19 (63.33)	22 (66.67)	0.782
Female	11 (36.67)	11 (33.33)	
Maximum diameter of the tumor			
≥5 cm	17 (62.96)	10 (37.04)	0.035
<5 cm	13 (36.11)	23 (63.89)	
TNM stage			
I-II	2 (6.67)	5 (15.15)	0.504
III-IV	28 (93.33)	28 (84.85)	
Degree of differentiation			
High	3 (10.0)	2 (6.06)	0.763
Intermediate	23 (76.67)	25 (75.76)	
Low	4 (13.33)	6 (18.18)	
Lymphatic metastasis			
Yes	26 (86.67)	27 (81.82)	0.599
No	4 (13.33)	6 (18.18)	
Distant metastasis			
Yes	6 (20.0)	11 (33.33)	0.234
No	24 (80.0)	22 (66.67)	
CEA			
<5 ng/mL	26 (86.67)	26 (78.79)	0.411
≥5 ng/mL	4 (13.33)	7 (21.21)	
CA-199			
<35 U/mL	26 (86.67)	26 (78.79)	0.411
≥35 U/mL	4 (13.33)	7 (21.21)	

CEA, carcinoembryonic antigen.

enchymal transition (EMT), which increases cell viability and invasion potential in cancer. Multiple studies have shown that the prognostic value of mesenchymal CTC (M-CTC) is higher than the total number of CTC, and it is positively correlated with cancer progression and low survival of patients [8, 9]. Therefore, breaking down the phenotypic characteristics of CTC can provide more information about CRC metastasis.

Research has suggested that the phenotype and molecular characteristics of CTC are valuable for CRC diagnosis and prognosis [10, 11]. For example, Fang et al. [11] found that CD133-positive CTC may be closely related to liver metastasis in CRC patients, but this study did not indicate whether CD133+ CTC could predict the survival of CRC patients. In patients with metastatic castration-sensitive prostate cancer, CD133-positive circulating tumor cells predict worse progression-free survival [12]. CD133 (prominin-1) is a cancer stem cell (CSCs) specific biomarker. CD133 is also expressed on many cancer cells, and CD133+ cells isolated from tumors can grow spherically for a long time or in xenograft models. CD133 can promote angiogenesis by activating the Wnt signaling pathway [13]. In CRC, the surface marker CD133 for CSCs is considered a target for drug therapy [14]. Therefore, exploring the expression of CTC by CD133 has significance for the prognosis of CRC patients. Based on the above evidence, we speculate that CD133-positive CTC can significantly predict the prognosis of CRC patients. To confirm this hypothesis, clinical data were prospectively collected from patients with colorectal cancer who underwent peripheral blood circulating tumor cytology.

In terms of CTC detection, many studies have confirmed that CanPatrol CTC enrichment technology is a breakthrough in conventional detection methods, and has high sensitivity for CTC detection and EMT typing identification. This technique can identify epithelial CTC (E-CTC), mesenchymal CTC (M-CTC), and mixed E/M-CTC, with a high detection rate and a more comprehensive detection of all types of CTC [15]. Based on this, in order to further explore the influence of CD133 expression in CTC on the prognosis of CRC, this study adopted CanPatrol® novel CTC typing detection technology to analyze the expression of CD133 in different subtypes of CTC and its correlation with prognosis.

Materials and methods

Study population

In this study, a retrospective analysis method was used to collect the clinical data of 63 patients with CRC who underwent peripheral

Table 2. Mesenchymal Circulating Tumor Cell (M-CTC) distribution and baseline characteristics of patients [n (%)]

	M-CTC		P
	≥1 (n=34)	<1 (n=29)	
Age (years)			
≥60	17 (50.0)	18 (62.07)	0.337
<60	17 (50.0)	11 (37.93)	
Gender			
Male	21 (61.76)	20 (68.97)	0.109
Female	13 (38.24)	9 (31.03)	
Maximum diameter of the tumor			
≥5 cm	12 (35.29)	15 (51.72)	0.189
<5 cm	22 (64.71)	14 (48.28)	
TNM stage			
I-II	1 (2.94)	6 (20.69)	0.067
III-IV	33 (97.06)	23 (79.31)	
Degree of differentiation			
High	2 (5.88)	3 (10.34)	0.473
Intermediate	25 (73.53)	23 (79.31)	
Low	7 (20.59)	3 (10.34)	
Lymphatic metastasis			
Yes	31 (91.18)	22 (75.86)	0.097
No	3 (8.82)	7 (24.14)	
Distant metastasis			
Yes	14 (82.35)	3 (17.65)	0.006
No	20 (43.48)	26 (56.52)	
CEA			
<5 ng/mL	27 (79.41)	25 (86.21)	0.479
≥5 ng/mL	7 (20.59)	4 (13.79)	
CA-199			
<35 U/mL	28 (82.35)	24 (82.76)	0.966
≥35 U/mL	6 (17.65)	5 (17.24)	

CEA, carcinoembryonic antigen

blood circulating tumor cytology in the Departments of Gastroenterology and Urology of Hunan Cancer Hospital from January 2016 to January 2021. Male to female ratio was 41 vs 22. Age range was 32 to 70 years old, average 59.73±10.76 years old. Inclusion criteria: Patients with pathologic diagnosis of colorectal cancer, and patients with complete clinical data. Exclusion criteria: Patients with malignant tumors at other sites, or patients who had received anti-cancer therapy or had their mass removed prior to admission. This study was approved by the Ethics Committee of the Affiliated Cancer Hospital of Xiangya Medical College, Central South University (Ethics number: KY2022305).

Data collection and follow-up

Age, maximum tumor diameter, tumor stage, degree of differentiation, lymph node metastasis (LNM), distant metastasis (DM), carcinoembryonic antigen (CEA), and cancer antigen 199 (CA-199), surgical options, chemotherapy regimen, CTC and CD133 detection results, progression-free survival (PFS) time, and overall survival (OS) time were gathered. All the patients were treated with surgery and chemotherapy. Chemotherapy regimens include 5-fluorouracil + folinic acid + oxaliplatin regimen (FOLFOX6), modified FOLFOX6 regimen (mFOLFOX6), and three-times weekly capecitabine and oxaliplatin regimen (XELOX). The follow-up was conducted from the time after diagnosis for enrolled patients. Follow-up included health examination, CEA monitoring, CA-199 monitoring, abdominal and/or pelvic ultrasound, chest X-ray, chest, abdominal and/or pelvic computerized tomography (CT) or magnetic resonance imaging (MRI), and colonoscopy. Follow-up time: Every 90 days after initial diagnosis and treatment until May 2022. Termination of follow-up: The follow-up deadline was reached, or contact was lost, or death was reported. Follow-up method: Patients returned regularly for follow-up according to each outpatient or hospitalization visit. Patients who failed to return to the clinic were followed up by telephone. The total follow-up rate was 96.0%. The main study indicators were progression-free survival (PFS) and overall survival (OS), and the formula for calculating OS was: OS = (number of surviving cases after n months of follow-up/number of cases at the beginning of follow-up) × 100%.

CTC detection and CD133 detection

CTC was detected by CanPatrol® CTC enrichment technique. The method consists of two main steps, first isolating CTC and then detecting EMT markers (EpCAM and vimentin) using RNA In Situ Hybridization (ISH). Specific steps: 5 ml of fasting venous blood was collected and

Table 3. Mixed Epithelial/Mesenchymal Circulating Tumor Cell (E/M-CTC) distribution and baseline characteristics of patients [n (%)]

	E/M-CTC		P
	≥1 (n=49)	<1 (n=14)	
Age (years)			
≥60	23 (46.94)	12 (85.71)	0.023
<60	26 (53.06)	2 (14.29)	
Gender			
Male	33 (67.35)	8 (57.14)	0.480
Female	16 (32.65)	6 (42.86)	
The maximum diameter of the tumor			
≥5 cm	19 (38.78)	8 (57.14)	0.221
<5 cm	30 (61.22)	6 (42.86)	
TNM stage			
I-II	5 (10.20)	2 (14.29)	0.668
III-IV	44 (89.80)	12 (85.71)	
Degree of differentiation			
High	3 (6.12)	2 (14.29)	0.608
Intermediate	38 (77.55)	10 (71.43)	
Low	8 (16.33)	2 (14.29)	
Lymphatic metastasis			
Yes	43 (87.76)	10 (71.43)	0.140
No	6 (12.24)	4 (28.57)	
Distant metastasis			
Yes	15 (88.26)	2 (14.29)	0.225
No	34 (73.91)	12 (85.71)	
CEA			
<5 ng/mL	40 (81.63)	12 (85.71)	0.565
≥5 ng/mL	9 (18.37)	2 (14.29)	
CA-199			
<35 U/mL	40 (81.63)	12 (85.71)	0.565
≥35 U/mL	9 (18.37)	2 (14.29)	

CEA, carcinoembryonic antigen.

placed into EDTA anticoagulant tube and stored at indoor temperature. Venous blood samples were treated with red blood cell lysis buffer (Solarbio, China) within 4 h of collection and filtered with 8 μm diameter pore calibration membranes (EMD Millipore) to enrich CTC. Then, the CTC were subjected to ISH with a combination of epithelial (EpCAM and CK8/CK18/CK19) and mesenchymal (Vimentin and Twist Family BHLH Transcription Factor 1 (TWIST1)) markers. Finally, the samples were stained with DAPI (Solarbio, China) for 5 min and analyzed with an automated imaging fluorescent microscope (EVOS FL Auto, Thermo Fisher Scientific). Finally, the CTC from each patient were grouped according to the identification of the markers. Patients were defined as CTC-positive if ≥1 CTC was observed per 5 ml of blood. The procedure before the detection of CD133 expression in CTC were the same as above. On the basis of CTC enrichment, CD133 mRNA probe was used, which was labeled purple by fluorescent labeling probe.

Outcome measures

First, the clinical features of colorectal cancer with different EMT types of CTC were compared. Then, the correlation between CD133 expressions of different CTC and clinical features of colorectal cancer was analyzed. Finally, the correlation between positive CD133 and progression-free survival in colorectal cancer patients was analyzed.

Statistical methods

SPSS 26.0 statistical software was used to process data. Student's t test was used to contrast the means of two groups, the rank-sum test was used to contrast the median of the two groups, and the one-way analysis of variance was used to contrast the means of three or more groups. Comparing the two groups: when the theoretical frequencies (T) ≥5, the χ^2 test was used, when 1≤T<5, the continuous corrected χ^2 test was used, and when the T<1, Fisher's exact test was used. Receiver operator characteristic curve (ROC) analysis was used to determine the best cutoff points of CTC and M-CTC for dividing patients into favorable and unfavorable prognostic groups based on CTC number and M-CTC number. The Kaplan-Meier analysis was used to establish PFS and OS models, and the log-rank test was used to contrast the survival rates of two groups. Multiple variate Cox regression was used to analyze the prognostic factors

Table 4. CD133 positive Epithelial Circulating Tumor Cells (E-CTC) and baseline characteristics of patients [n (%)]

	CD133 positive E-CTC		P
	≥1 (n=22)	<1 (n=41)	
Age (years)			
≥60	16 (72.73)	19 (46.34)	0.045
<60	6 (27.27)	22 (53.66)	
Gender			
Male	14 (63.64)	27 (65.85)	0.860
Female	8 (36.36)	14 (34.15)	
The maximum diameter of the tumor			
≥5 cm	13 (59.09)	14 (34.15)	0.056
<5 cm	9 (40.91)	27 (65.85)	
TNM stage			
I-II	2 (9.09)	5 (12.20)	0.709
III-IV	20 (90.91)	36 (87.80)	
Degree of differentiation			
High	3 (13.64)	2 (4.88)	0.462
Intermediate	16 (72.72)	32 (78.05)	
Low	3 (13.64)	7 (17.07)	
Lymphatic metastasis			
Yes	18 (81.82)	35 (85.37)	0.713
No	4 (18.18)	6 (14.63)	
Distant metastasis			
Yes	3 (13.64)	14 (34.15)	0.080
No	19 (83.36)	27 (65.85)	
CEA			
<5 ng/mL	18 (81.82)	34 (82.93)	0.912
≥5 ng/mL	4 (18.18)	7 (17.07)	
CA-199			
<35 U/mL	18 (81.82)	34 (82.93)	0.912
≥35 U/mL	4 (18.18)	7 (17.07)	

CEA, carcinoembryonic antigen.

of patients with colorectal cancer. P<0.05 was deemed significant. Cox regression model, also known as proportional risk regression model, is a semi-parametric regression model proposed by British statistician D.R. Cox in 1972. The model can be used to describe the influence of multiple characteristics over time on mortality at a certain time. This model takes survival outcome and survival time as dependent variables, and then analyzes the influence of many factors on survival time, by a typical multi-factor analysis method. Cox proportional risk regression model can be expressed as:

$$h(t|X) = h_0(t) * exp(\beta_1 x_1 + \beta_2 x_2 + ... + \beta_n x_n)$$

Results

Correlation between different EMT types of CTC and clinical factors of CRC

CTC was detected in all patients, and the positive rate of E-CTC was 47.62% (30/63), M-CTC was 53.97% (34/63) and mixed E/M-CTC was 77.78% (49/63). The positive rate of E-CTC in patients with tumor diameter ≥5 cm was significantly higher than that in patients with tumor diameter <5 cm (P=0.035, **Table 1**). The positive rate of M-CTC in patients with diabetes was higher than that in patients without diabetes (P=0.006, **Table 2**). There was no significant difference in the positive rate of mixed E/M-CTCs among different maximum tumor diameters, tumor stages, LNM, DM, CEA, and CA-199 levels (all P>0.05, **Table 3**).

Correlation between CD133 expression of different CTC phenotypes and clinical factors in CRC

There was no significant difference in CD133-positive E-CTC among different tumor diameters, tumor stage, LNM, DM, CEA expression, and CA-199 expression levels (all P>0.05, **Table 4**). The CD133-positive M-CTCs were significantly higher in DM and CEA>5 ng/mL patients than in non-DM and CEA≤5 ng/mL patients (P<0.001, P=0.019, as shown in **Table 5**). CD133-positive mixed E/M-CTC were not correlated with maximum tumor diameter, tumor stage, LNM, DM, CEA expression, or CA-199 expression (all P>0.05, **Table 6**).

CD133 positive M-CTC predicts distant metastasis

The AUC of M-CTC for predicting DM was 0.808 (95% CI=0.671-0.944), P<0.001, cutoff point =2.5. The AUC of CD133-positive M-CTC for predicting lymph node metastasis was 0.878

Table 5. CD133-positive Mesenchymal Circulating Tumor Cells (M-CTC) and baseline characteristics of patients [n (%)]

	CD133 positive M-CTC		P
	≥1 (n=21)	<1 (n=42)	
Age (years)			
≥60	8 (38.10)	27 (64.29)	0.049
<60	13 (61.90)	15 (35.71)	
Gender			
Male	12 (57.14)	29 (69.05)	0.350
Female	9 (42.86)	13 (30.95)	
The maximum diameter of the tumor			
≥5 cm	8 (38.10)	19 (45.24)	0.589
<5 cm	13 (61.90)	23 (54.76)	
TNM stage			
I-II	1 (4.76)	6 (14.29)	0.257
III-IV	20 (95.24)	36 (85.71)	
Degree of differentiation			
High	0 (0)	5 (11.90)	0.186
Intermediate	16 (76.19)	32 (76.20)	
Low	5 (23.81)	5 (11.90)	
Lymphatic metastasis			
Yes	18 (85.71)	35 (83.33)	0.807
No	3 (14.29)	7 (16.67)	
Distant metastasis			
Yes	14 (82.35)	3 (17.65)	<0.001
No	7 (15.22)	39 (84.78)	
CEA			
<5 ng/mL	14 (33.33)	38 (66.67)	0.019
≥5 ng/mL	7 (63.64)	4 (36.36)	
CA-199			
<35 U/mL	15 (71.43)	37 (88.10)	0.100
≥35 U/mL	6 (28.57)	5 (11.90)	

CEA, carcinoembryonic antigen.

(95% CI=0.761-0.995), P<0.001, cutoff point =0.5; **Figure 1**.

Correlation between CD133 expression and PFS in patients with CRC

55 patients were followed up, and the follow-up rate was 87.30%. The median follow-up time was 14 months. In the follow-up period, 19 patients had disease progression and 5 patients died. According to the cutoff point obtained by ROC analysis, the PFS of M-CTC>2.5/5 ml group (0%) was lower than that of M-CTC≤2.5/5 mL group (76.5%), P<0.05. The PFS of CD133 positive M-CTC>0.5/5 ml group (18.6%) was lower than that of the CD133 positive M-CTC≤0.5/5 mL group (76.5%), P<0.05. No difference was found in OS between the CD133 positive M-CTC>0.5/5 mL group (71.7%) and the CD133 positive M-CTC≤0.5/5 ml group (93.8%), P=0.054, **Figure 2**. Cox multivariate regression analysis showed that M-CTC and CD133 positive M-CTC were not risk factors for progression-free survival and overall survival in patients with colorectal cancer (all P>0.05, as shown in **Tables 7**, **8**).

Discussion

According to global cancer statistics in 2020, there were 560,000 new cases of CRC and 290,000 deaths in China [1]. With the continuous development of medical technology, the diagnosis rate and treatment effect of CRC have significantly improved. However, the rate of distant metastasis is relatively high in patients with CRC, and the 5-year survival rate is reduced in patients with distant metastasis [16]. Among patients with CRC undergoing radical surgery, 20%-45% are still likely to have recurrence or DM [17]. Thus, distant metastasis was considered to be a vital prognostic factor for CRC and a major cause of cancer-related death in CRC patients [18]. Therefore, early and precise prediction of cancer metastasis is valuable for improving the prognosis of CRC patients.

At present, auxiliary diagnosis, efficacy evaluation and prognostic monitoring methods of CRC mainly include detection of serum tumor markers [such as CEA and CA-199], imaging examination [such as CT, PET-CT, MRI, ultrasound], endoscopy, and pathologic biopsy. However, the above examination methods have some shortcomings. For example, the increase in CEA level indicates the existence of malignant tumors such as gastrointestinal tumor, urinary

Table 6. CD133 positive Epithelial/Mesenchymal Circulating Tumor Cells (E/M-CTC) and baseline characteristics of patients [n (%)]

	CD133 positive E/M-CTC		P
	≥1 (n=44)	<1 (n=19)	
Age (years)			
≥60	20 (45.45)	15 (78.95)	0.014
<60	24 (54.55)	4 (21.05)	
Gender			
Male	30 (68.18)	11 (57.89)	0.432
Female	14 (31.82)	8 (42.11)	
The maximum diameter of the tumor			
≥5 cm	17 (38.64)	10 (52.63)	0.303
<5 cm	27 (61.36)	9 (47.37)	
TNM stage			
I-II	5 (11.36)	2 (10.53)	0.923
III-IV	39 (88.64)	17 (89.47)	
Degree of differentiation			
High	3 (6.82)	2 (10.53)	0.881
Intermediate	34 (77.27)	14 (73.68)	
Low	7 (15.91)	3 (15.79)	
Lymphatic metastasis			
Yes	38 (86.36)	15 (78.95)	0.460
No	6 (13.64)	4 (21.05)	
Distant metastasis			
Yes	15 (34.09)	2 (10.53)	0.053
No	29 (65.91)	17 (89.47)	
CEA			
<5 ng/mL	35 (79.55)	17 (89.47)	0.341
≥5 ng/mL	9 (20.45)	2 (10.53)	
CA-199			
<35 U/mL	35 (79.55)	17 (89.47)	0.341
≥35 U/mL	9 (20.45)	2 (10.53)	

CEA, carcinoembryonic antigen.

tract tumor, breast carcinoma, and lung carcinoma, but an increase in CEA level also occurs in benign lesions such as colitis and pancreatitis. In addition, the serum CEA level of patients with chronic illness such as cardiovascular and cerebrovascular diseases and diabetes mellitus is increased [19], indicating that the specificity of CEA is not high. Although imaging examinations such as CT, MRI, PET-CT, and endoscopy can visually display tumor lesions, they cannot find occult, metastatic or small postoperative residual lesions without a time lag. Tissue biopsy is the gold standard for the diagnosis of CRC, but tissue biopsy has the possibility of repeated sampling, more trauma, difficult sampling of special sites, some false negatives, and inability to dynamically monitor. Therefore, it is urgent to find a biological index that can detect tumor early, monitor efficacy dynamically, and evaluate prognosis.

It was found that after EMT, the protein expression of the original epithelial phenotype was down-regulated, while the protein expression of the mesenchymal phenotype was up-regulated. In other words, cells suffered loss of cell polarity, thus enhancing the transport capacity of cells, making them shed from the original tumor tissue or enter the circulatory system to form CTC, laying the foundation for distant metastasis of tumors [20]. To date, in several studies, CTC have been considered a potential noninvasive diagnostic and prognostic marker for metastatic CRC. A meta-analysis published in 2013 [21] showed that CTC positive mCRC patients had poor OS and PFS. Similarly, Connor et al. [22] demonstrated that hepatic venous chamber CTC count in patients with CRC liver metastasis was associated with poor DFS and OS. In addition, Wu et al. [23] compared the correlation between CTC types and tumor metastasis, and the results demonstrated that M-CTC type was more strongly related to tumor metastasis than other CTC types. Likewise, a study by Zhong et al. [24] demonstrated that M-CTC were correlated with tumor size, TNM stage, vascular invasion and CEA, and M-CTC were related to poor DFS. A meta-analysis published in 2018 [25] demonstrated that CTC were highly related to worse survival and invasive disease progression. In addition, subgroup analysis showed that CTC-positive patients also had worse PFS and OS. In this study, it was found that interstitial CTCS were correlated

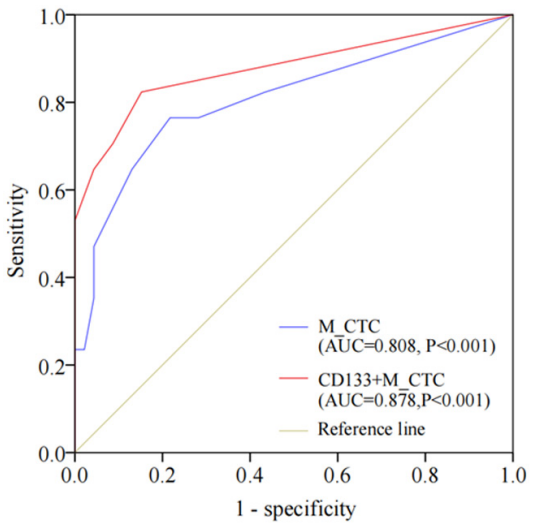

Figure 1. Receiver operator characteristic curve (ROC). The red line is the number of CD133-positive mesenchymal circulating tumor cells (CD133+ M-CTC), while the blue line is the number of mesenchymal circulating tumor cells (M-CTC).

with distant metastasis in colorectal cancer patients, that is, the positive expression rate of M-CTC in patients with distant metastasis was higher than that in patients without distant metastasis, which was consistent with previous studies. The positive expression rate of M-CTC increased with the progression of colorectal cancer. In addition, the progression-free survival rate in patients with >2.5/5 ml M-CTCs was significantly lower than that of patients with ≤2.5/5 ml M-CTCs, suggesting that M-CTCs may be important for prognostic evaluation of patients with colorectal cancer.

CD133 is one of the most characteristic biomarkers for the isolation of CSCs and is considered a target for the treatment of CRC [26]. In order to further analyze the expression of CD133 in CTC and the effect of CD133 expression on the prognosis of CRC, this research detected the positive expression of CD133. The results showed that CD133 positive M-CTC were closely related to distant metastasis and CEA expression in CRC patients. The PFS of patients with CD133 positive M-CTC>0.5/5 ml (18.6%) was lower than that of patients ≤0.5/5 ml (76.5%). In a study of the clinical significance of CD133, CD24, and CD44 RNA expression in CRC, it was found that high CD133-/low CD24- tumor had worse disease-specific survival and OS, and higher rate of relapse. Li et al. [27] found that CD133 knockout reduced epithelial-mesenchymal transformation and finally inhibited the invasion of colon cancer, which may be an effective treatment for colon cancer. These results indicated that positive CD133 expression could be used as a prognostic marker for CRC.

Cox multivariate regression analysis showed that M-CTC and CD133 positive M-CTC were not risk factors for progression-free survival and overall survival in patients with colorectal cancer. Apossible reason is that the sample size required for risk factor analysis is large, while the sample size in this study is small, and the results obtained may be different from the results of Kaplan-Meier analysis. Therefore, the value of CTC in clinical characteristics still needs to be further verified through prospective, large sample and multicenter clinical studies.

Conclusion

This study preliminarily suggests that M-CTC is related to distant node metastasis in patients. CD133 positive M-CTC is associated with PFS and can be used as a prognostic indicator for patients. Due to the limitations of the research time of this project, only short-term follow-up records were recorded. If time permits in the future, we will further expand the sample size and long-term follow-up time.

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Disclosure of conflict of interest

None.

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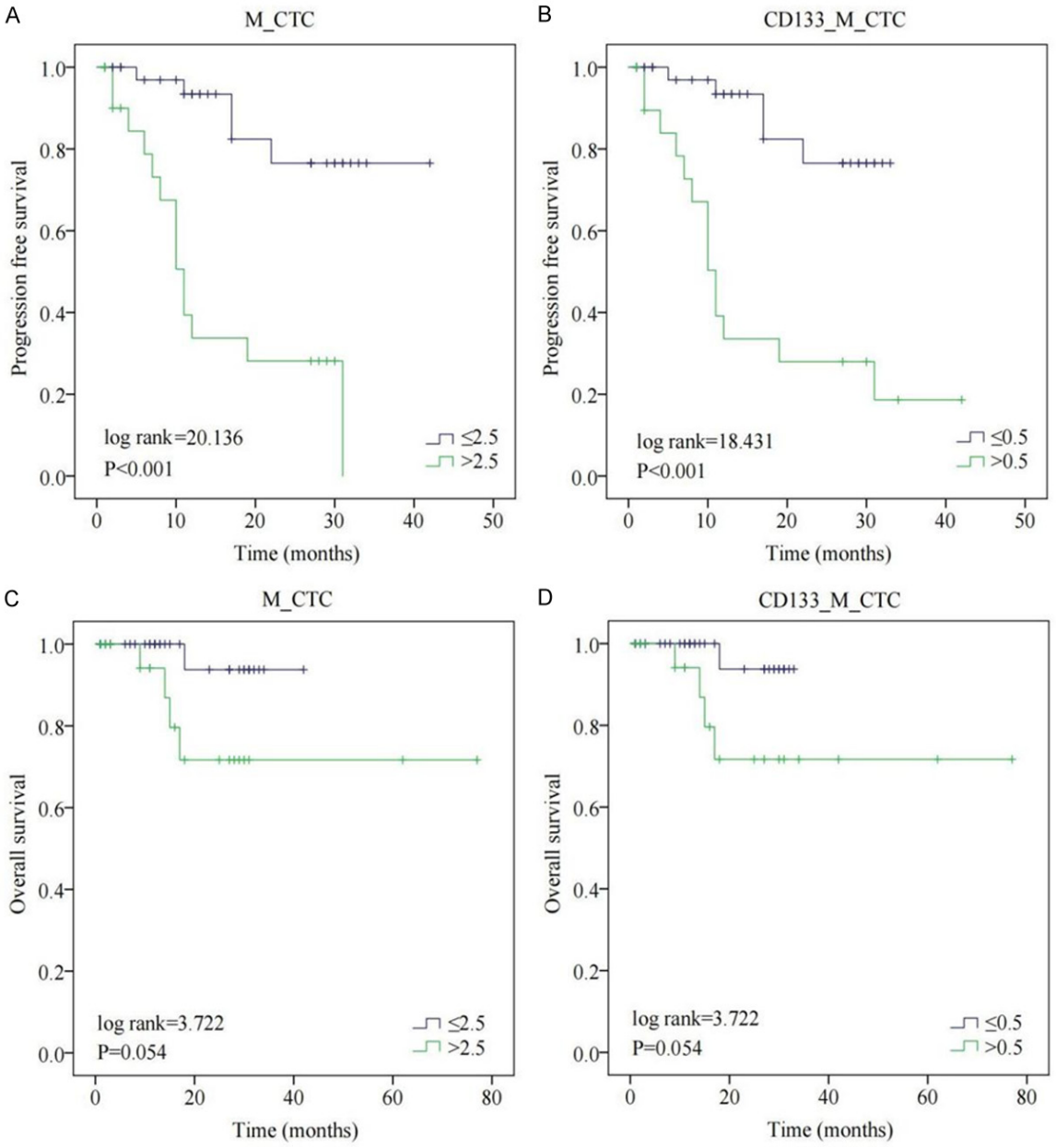

Figure 2. Survival analysis: A: Comparison of progression-free survival between patients with mesenchymal circulating tumor cells (CTC) >2.5/5 ml and mesenchymal CTC≤2.5/5 ml; B: Comparison of progression-free survival between CD133-positive mesenchymal CTC>0.5/5 ml and CD133-positive mesenchymal CTC≤0.5/5 ml; C: Comparison of overall survival between patients with mesenchymal CTC>2.5/5 ml and mesenchymal CTC≤2.5/5 ml; D: Comparison of overall survival between CD133-positive mesenchymal CTC patients >0.5/5 ml and CD133-positive mesenchymal CTC≤0.5/5 ml patients.

Table 7. Cox regression analysis of progression-free survival

	B	S.E.	Walds	df	P	Exp (B)	95.0% CI lower	95.0% CI upper
M-CTC	1.202	0.913	1.734	1	0.188	3.326	0.556	19.901
CD133 positive M-CTC	0.400	0.968	0.170	1	0.680	1.491	0.224	9.945

Notes: M-CTC is Mesenchymal Circulating Tumor Cells.

Table 8. Cox regression analysis of overall survival

	B	S.E.	Walds	df	P	Exp (B)	95.0% CI lower	95.0% CI upper
M-CTC	1.045	1.050	0.991	1	0.319	2.845	0.363	22.278
CD133 positive M-CTC	-0.750	1.114	0.453	1	0.501	0.473	0.053	4.193

Notes: M-CTC is Mesenchymal Circulating Tumor Cells.

References

[1] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021; 71: 209-249.

[2] Chen J, Wu Y, Luo X, Jin D, Zhou W, Ju Z, Wang D, Meng Q, Wang H, Fu X, Xu J and Song Z. Circular RNA circRHOBTB3 represses metastasis by regulating the HuR-mediated mRNA stability of PTBP1 in colorectal cancer. Theranostics 2021; 11: 7507-7526.

[3] Alix-Panabieres C and Pantel K. Liquid biopsy: from discovery to clinical application. Cancer Discov 2021; 11: 858-873.

[4] Schuster E, Taftaf R, Reduzzi C, Albert MK, Romero-Calvo I and Liu H. Better together: circulating tumor cell clustering in metastatic cancer. Trends Cancer 2021; 7: 1020-1032.

[5] Yu T, Wang C, Xie M, Zhu C, Shu Y, Tang J and Guan X. Heterogeneity of CTC contributes to the organotropism of breast cancer. Biomed Pharmacother 2021; 137: 111314.

[6] Ye Q, Ling S, Zheng S and Xu X. Liquid biopsy in hepatocellular carcinoma: circulating tumor cells and circulating tumor DNA. Mol Cancer 2019; 18: 114.

[7] Yang J, Cheng S, Zhang N, Jin Y and Wang Y. Liquid biopsy for ovarian cancer using circulating tumor cells: recent advances on the path to precision medicine. Biochim Biophys Acta Rev Cancer 2022; 1877: 188660.

[8] Hou J, Guo C and Lyu G. Clinical significance of epithelial-mesenchymal transition typing of circulating tumour cells in colorectal cancer. Colorectal Dis 2020; 22: 581-587.

[9] Menyailo ME, Bokova UA, Ivanyuk EE, Khozyainova AA and Denisov EV. Metastasis prevention: focus on metastatic circulating tumor cells. Mol Diagn Ther 2021; 25: 549-562.

[10] Lieto E, Galizia G, Orditura M, Romano C, Zamboli A, Castellano P, Mabilia A, Auricchio A, DE Vita F and Gemei M. CD26-positive/CD326-negative circulating cancer cells as prognostic markers for colorectal cancer recurrence. Oncol Lett 2015; 9: 542-550.

[11] Fang C, Fan C, Wang C, Huang Q, Meng W, Yu Y, Yang L, Peng Z, Hu J, Li Y, Mo X and Zhou Z. CD133+CD54+CD44+ circulating tumor cells as a biomarker of treatment selection and liver metastasis in patients with colorectal cancer. Oncotarget 2016; 7: 77389-77403.

[12] Yang Y, Liu Z, Wang Q, Chang K, Zhang J, Ye D, Kong Y and Dai B. Presence of CD133-positive circulating tumor cells predicts worse progression-free survival in patients with metastatic castration-sensitive prostate cancer. Int J Urol 2022; 29: 383-389.

[13] Barzegar Behrooz A, Syahir A and Ahmad S. CD133: beyond a cancer stem cell biomarker. J Drug Target 2019; 27: 257-269.

[14] Akbari M, Shomali N, Faraji A, Shanehbandi D, Asadi M, Mokhtarzadeh A, Shabani A and Baradaran B. CD133: an emerging prognostic factor and therapeutic target in colorectal cancer. Cell Biol Int 2020; 44: 368-380.

[15] Li J, Liao Y, Ran Y, Wang G, Wu W, Qiu Y, Liu J, Wen N, Jing T, Wang H and Zhang S. Evaluation of sensitivity and specificity of CanPatrol technology for detection of circulating tumor cells in patients with non-small cell lung cancer. BMC Pulm Med 2020; 20: 274.

[16] Siegel RL, Miller KD and Jemal A. Cancer statistics, 2018. CA Cancer J Clin 2018; 68: 7-30.

[17] Jeon J, Du M, Schoen RE, Hoffmeister M, Newcomb PA, Berndt SI, Caan B, Campbell PT, Chan AT, Chang-Claude J, Giles GG, Gong J, Harrison TA, Huyghe JR, Jacobs EJ, Li L, Lin Y, Le Marchand L, Potter JD, Qu C, Bien SA, Zubair N, Macinnis RJ, Buchanan DD, Hopper JL, Cao Y, Nishihara R, Rennert G, Slattery ML, Thomas DC, Woods MO, Prentice RL, Gruber SB, Zheng Y, Brenner H, Hayes RB, White E, Peters U and Hsu L; Colorectal Transdisciplinary Study and Genetics and Epidemiology of Colorectal Cancer Consortium. Determining risk of colorectal cancer and starting age of screening based on lifestyle, environmental, and genetic factors. Gastroenterology 2018; 154: 2152-2164, e2119.

[18] Filip S, Vymetalkova V, Petera J, Vodickova L, Kubecek O, John S, Cecka F, Krupova M, Manethova M, Cervena K and Vodicka P. Distant metastasis in colorectal cancer patients-do we have new predicting clinicopathological and molecular biomarkers? A comprehensive review. Int J Mol Sci 2020; 21: 5255.

[19] Hao C, Zhang G and Zhang L. Serum CEA levels in 49 different types of cancer and noncancer

diseases. Prog Mol Biol Transl Sci 2019; 162: 213-227.
[20] Singh M, Yelle N, Venugopal C and Singh SK. EMT: mechanisms and therapeutic implications. Pharmacol Ther 2018; 182: 80-94.
[21] Groot Koerkamp B, Rahbari NN, Buchler MW, Koch M and Weitz J. Circulating tumor cells and prognosis of patients with resectable colorectal liver metastases or widespread metastatic colorectal cancer: a meta-analysis. Ann Surg Oncol 2013; 20: 2156-2165.
[22] Connor AA, McNamara K, Al-Sukhni E, Diskin J, Chan D, Ash C, Lowes LE, Allan AL, Zogopoulos G, Moulton CA and Gallinger S. Central, but not peripheral, circulating tumor cells are prognostic in patients undergoing resection of colorectal cancer liver metastases. Ann Surg Oncol 2016; 23: 2168-2175.
[23] Wu F, Zhu J, Mao Y, Li X, Hu B and Zhang D. Associations between the epithelial-mesenchymal transition phenotypes of circulating tumor cells and the clinicopathological features of patients with colorectal cancer. Dis Markers 2017; 2017: 9474532.
[24] Zhong Y, Ma T, Qiao T, Hu H, Li Z, Luo K, Wang Y, Tang Q, Wang G, Huang R and Wang X. Role of phenotypes of circulating tumor cells in the diagnosis and treatment of colorectal cancer. Cancer Manag Res 2021; 13: 7077-7085.
[25] Tan Y and Wu H. The significant prognostic value of circulating tumor cells in colorectal cancer: a systematic review and meta-analysis. Curr Probl Cancer 2018; 42: 95-106.
[26] Catalano V, Di Franco S, Iovino F, Dieli F, Stassi G and Todaro M. CD133 as a target for colon cancer. Expert Opin Ther Targets 2012; 16: 259-267.
[27] Li W, Cho MY, Lee S, Jang M, Park J and Park R. CRISPR-Cas9 mediated CD133 knockout inhibits colon cancer invasion through reduced epithelial-mesenchymal transition. PLoS One 2019; 14: e0220860.