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RESEARCH ARTICLE

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The relation of blood cell division control protein 42 level with disease risk, comorbidity, tumor features/markers, and prognosis in colorectal cancer patients

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Abstract

Background: Cell division control protein 42 (CDC42) is involved in colorectal cancer (CRC) progression by modulating $CD8^+$ T cell activation, immune escape, and direct oncogenetic biological processes. This study aimed to explore the correlation of blood CDC42 with disease risk, comorbidities, disease features, tumor markers, and prognosis among CRC patients.

Methods: CDC42 in peripheral blood mononuclear cells was detected by reverse transcription-quantitative polymerase chain reaction from 250 resectable CRC patients and 50 healthy controls (HCs). CDC42 was divided by quartiles, as well as high and low expressions in CRC patients for correlation and survival analysis.

Results: CDC42 was elevated in CRC patients vs. HCs ($p < 0.001$), which had a good ability to distinguish CRC patients from HCs with the area under the curve (95% confidence interval) of 0.889 (0.841–0.937). In CRC patients, CDC42 was not associated with demographics or comorbidities (all $p > 0.05$), while its higher quartile was linked to increased T stage ($p < 0.001$), N stage ($p = 0.009$), TNM stage ($p < 0.001$), abnormal carcinoembryonic antigen ($p = 0.043$), and adjuvant chemotherapy administration ($p = 0.002$). Higher CDC42 quartile ($p = 0.002$) and CDC42 high (vs. low) ($p < 0.001$) were related to worse disease-free survival (DFS); meanwhile, elevated CDC42 quartile ($p = 0.002$) and CDC42 high (vs. low) ($p = 0.001$) were also linked to poor overall survival (OS). Multivariate Cox's regression analysis presented that CDC42 quartile 3 and 4 (vs. quartile 1) independently predicted declined DFS and OS (all $p < 0.05$).

Conclusion: Circulating CDC42 relates to higher disease risk, T, N, and TNM stage, abnormal tumor marker, and poor prognosis among CRC patients.

KEYWORDS
blood CDC42, colorectal cancer, DFS, disease characteristics, OS

1 | INTRODUCTION

Colorectal cancer (CRC) is one of the most prevalent malignancies and the second leading cause of cancerous deaths in 2020, with hyperlipidemia, obesity, and alcohol consumption as its main risk factors.[1–3] Currently, many CRC patients are diagnosed at an early stage due to the development of screening by colonoscopy, while some heterogeneous tumors may be ignored by this procedure; besides, coloscopy is invasive and discomfortable, which may induce harm to patients.[4,5] Meanwhile, a proportion of CRC patients do not receive optimal treatment partly due to lacking reliable predictive factors for prognosis, which leads to dismal clinical outcomes among patients.[6,7] Thus, the exploration of convenient, accessible, and reliable biomarkers is imperative to improving the management of CRC patients.

Cell division control protein 42 (CDC42), a small GTPase belonging to the Rho family, plays an important role in regulating several crucial tumor biological processes through modulating actin cytoskeleton remodeling, cell adhesion, cell motility, vesicle transport, transcriptional activation, gene expression, and cell cycle regulation.[8,9] For instance, CDC42 is involved in tumor acceleration via suppressing $CD8^+$ T cells[10,11]; meanwhile, CDC42 has the capacity of promoting the immune escape of tumor cells[12,13]; moreover, CDC42 is also able to directly regulate malignant functions of CRC cells, including promoting proliferation, migration, and invasion.[14–16] Therefore, the above-mentioned data present that CDC42 might take part in tumorigenesis and progression of CRC. Interestingly, it has been reported that CDC42 is related to a higher risk of breast cancer[17]; it is also linked to unfavorable survival among lung cancer patients.[18] Taken together, we deduced that blood CDC42 might serve as a convenient and available biomarker for CRC diagnosis and prognosis, while related data is scarce.

Therefore, the present study aimed to explore the association of blood CDC42 with disease risk, comorbidities, tumor features/markers, and prognosis among CRC patients.

2 | METHODS

2.1 | Participants

A total of 250 resectable first-ever CRC patients who underwent surgical resection between January 2017 and December 2020 were consecutively enrolled. The inclusion criteria for CRC patients: (1) histologically confirmed primary CRC; (2) resectable CRC; and (3) aged ≥18 years. The exclusion criteria for CRC patients: (1) had distant metastases; (2) had other malignancies; (3) accompanied with autoimmune diseases or hematological malignancies; (4) history of CRC; and (5) pregnant or nursing women. Besides, 50 age- and sex-matched people were also enrolled as healthy controls (HCs). The inclusion criteria for HCs were (1) aged ≥18 years and (2) proved good health by physical examination in our hospital. The exclusion criteria for HCs were the same as for CRC patients. The study was approved by the Ethical Committee of our hospital. The written informed consent was provided by each subject.

2.2 | Clinical data

After enrollment, we collected the demographics, comorbidities, tumor features, tumor marker, and adjuvant treatment information of patients using case report form. The demographics included the followings: age; gender (female or male); and smoker (yes/no). The comorbidities included the followings: hypertension (yes/no); hyperlipidemia (yes/no); and diabetes (yes/no). The tumor features included the followings: diagnosis (colon or rectum); Eastern Cooperative Oncology Group Performance Status (ECOG PS); tumor differentiation (well or moderate or poor); tumor size; T/N/M/TNM stage. The tumor marker included carcinoembryonic antigen (CEA), and CEA ≥5 ng/mL was considered CEA abnormal. The treatment information included the followings: adjuvant chemotherapy (yes/no) and adjuvant regimen (Capecitabine or capecitabine plus oxaliplatin). After 2 weeks of adjuvant chemotherapy, the drug was discontinued for 1 week, and 3 weeks were used as a cycle of treatment, for a total of 8 cycles of treatment. Besides, the age and gender of HCs were also recorded.

2.3 | Blood sample collection

The peripheral blood (PB) samples of CRC patients were collected by venipuncture within 24 h after admission. The PB samples of HCs were also collected during physical examination.

After collection, peripheral blood mononuclear cells (PBMCs) were isolated from PB samples by Ficoll-Paque density gradient centrifugation (2500 revolutions per minute, 30 min, without brake).

2.4 | RT-qPCR assay

RT-qPCR assay was carried out for quantitative analysis of the expression of CDC42 in the PBMCs. In brief, total RNA was extracted by QIAamp RNA Blood Mini Kit (Qiagen) and reversely transcribed by iScript™ cDNA Synthesis Kit (Bio-Rad). Afterward, qPCR was performed by TB Green™ Fast qPCR Mix (Takara). After incubated at 95°C for 5 min, the qPCR was performed by 30 cycles of 95°C for 30 sec, 55°C for 1 min, and 70°C for 1 min. Among these, GAPDH was used as the internal reference. Specific primers for qPCR were as follows: primers for CDC42, forward: 5'-CCATCGGAATATGTACCGACTG-3', reverse: 5'-CTCAGCGGTCGTAATCTGTCA-3'. GAPDH was used as an internal control. Primers for GAPDH, forward: 5'-GAGTCCACTGGCGTCTTCAC-3', reverse: 5'-ATCTTGAGGCTGTTGTCATACTTCT-3'. The expression of CDC42 was calculated by the $2^{-\Delta\Delta Ct}$ method.[19]

2.5 | Follow-up

After tumor resection, CRC patients were regularly followed up. The follow-up plan was 1–2 months for the first half year, 3–6 months for the next half year and every 6 months from the second year. Disease-free survival (DFS) and overall survival (OS) were estimated by the follow-up records of CRC patients. In survival analyses, thirty-eight CRC patients who lost to follow-up were processed as censored data.

2.6 | Statistics

The 25th, 50th, and 75th percentile of CDC42 in CRC patients were 2.064, 2.641, and 3.906, respectively. In association analysis, CDC42 in CRC patients was classified as quartile 1 (≤25th percentile), quartile 2 (25th-50th percentile), quartile 3 (50th-75th percentile), and quartile 4 (>75th percentile). In survival analysis, CDC42 in CRC patients was divided as low expression (≤50th percentile) and high expression (>50th percentile).

The comparisons of CDC42 between different subjects were assessed using Mann–Whitney *U* test. The profile of CDC42 in differentiating subjects was estimated using the receiver-operating characteristic (ROC) curve. The associations between CDC42 quartiles and demographics, comorbiditiestd, tumor features or adjuvant treatment information were evaluated using the Mantel–Haenszel Chi-square test or Chi-square test.

The correlation of CDC42 (high or low) with DFS and OS was examined by the Kaplan–Meier method and Log-rank test. The prognostic value was estimated using Cox's proportional hazard regression model analysis with forward-stepwise mode. A p value <0.05 indicated a statistical significance. The statistical analyses were performed using SPSS V.26.0 (IBM Corp.). The figures were plotted using GraphPad Prism V.7.02 (GraphPad Software Inc.).

3 | RESULTS

3.1 | Baseline characteristics of CRC patients

Among 250 CRC patients, the mean age was 63.0 ± 10.9 years; besides, there were 89 (35.6%) females and 161 (64.4%) males. Furthermore, there were 79 (31.6%), 39 (15.6%), and 33 (13.2%) patients with hypertension, hyperlipidemia, and diabetes, respectively. Moreover, there were 132 (52.8%) and 118 (47.2%) patients with ECOG PS scores of 0 and 1, respectively. Additionally, 46 (18.4%), 117 (46.8%), and 87 (34.8%) patients possessed well, moderate, and poor tumor differentiation, accordingly. Meanwhile, there were 33 (13.2%), 121 (48.4%), and 96 (38.4%) patients with TNM stages of I, II, and III, respectively. In addition, 196 (78.4%) patients received adjuvant chemotherapy, among which 48 (19.2%) patients were administrated with capecitabine and 148 (59.2%) received capecitabine plus oxaliplatin (Table 1).

TABLE 1 Baseline characteristics of CRC patients

Items	CRC patients ($N = 250$)
Demographics	
Age (years), mean ± SD	63.0 ± 10.9
Gender, *n* (%)	
Female	89 (35.6)
Male	161 (64.4)
Smoker, *n* (%)	78 (31.2)
Comorbidities	
Hypertension, *n* (%)	79 (31.6)
Hyperlipidemia, *n* (%)	39 (15.6)
Diabetes, *n* (%)	33 (13.2)
Tumor features	
Diagnosis, *n* (%)	
Colon	169 (67.6)
Rectum	81 (32.4)
ECOG PS, *n* (%)	
0	132 (52.8)
1	118 (47.2)
Tumor differentiation, *n* (%)	
Well	46 (18.4)
Moderate	117 (46.8)
Poor	87 (34.8)
Tumor size, *n* (%)	
<5 cm	165 (66.0)
≥5 cm	85 (34.0)
T stage, *n* (%)	
T1	6 (2.4)
T2	27 (10.8)
T3	214 (85.6)
T4	3 (1.2)
N stage, *n* (%)	
N0	154 (61.6)
N1	64 (25.6)
N2	32 (12.8)
M stage, *n* (%)	
M0	250 (100.0)
TNM stage, *n* (%)	
Stage I	33 (13.2)
Stage II	121 (48.4)
Stage III	96 (38.4)
Tumor marker	
CEA, *n* (%)	
Normal (<5 ng/mL)	149 (59.6)
Abnormal (≥5 ng/mL)	101 (40.4)

(Continues)

TABLE 1 (Continued)

Items	CRC patients (*N* = 250)
Adjuvant treatment information	
Adjuvant chemotherapy, *n* (%)	
No	54 (21.6)
Yes	196 (78.4)
Adjuvant regimen, *n* (%)	
Capecitabine	48 (19.2)
CapeOx	148 (59.2)

Abbreviations: CapeOX, capecitabine plus oxaliplatin; CEA, carcinoembryonic antigen; CRC, colorectal cancer; ECOG PS, Eastern Cooperative Oncology Group Performance Status; SD, standard deviation.

3.2 | Comparison of CDC42 between CRC patients and HCs

CDC42 was elevated in CRC patients compared to HCs (median (interquartile range): 2.641 (2.064–3.906) vs. 0.992 (0.790–1.591), $p < 0.001$) (Figure 1A). Moreover, CDC42 had a good ability to discriminate CRC patients from HCs with an area under the curve (95% confidence interval) of 0.889 (0.841–0.937); meanwhile, CDC42 was 1.891 at the best cut-off point with sensitivity of 81.2% and specificity of 84.0%, respectively (Figure 1B).

3.3 | Correlation of CDC42 with demographics, comorbidities, tumor features, tumor marker, and adjuvant treatment information in CRC patients

CDC42 in CRC patients was classified as quartile 1 (≤25th percentile), quartile 2 (25th-50th percentile), quartile 3 (50th-75th percentile), and quartile 4 (>75th percentile) in association analysis, which revealed that no correlation was found in CDC42 quartile with age, gender, smoking, hypertension, hyperlipidemia, or diabetes (all $p > 0.05$; Table 2). In addition, elevated CDC42 quartile was linked to increased T stage ($p < 0.001$), N stage ($p = 0.009$), TNM stage ($p < 0.001$), abnormal carcinoembryonic antigen (CEA; $p = 0.043$), and adjuvant chemotherapy ($p = 0.002$), while CDC42 quartile was not correlated with diagnosis as colon cancer or rectum cancer, ECOG PS score, tumor differentiation, tumor size, or adjuvant regimen (all $p > 0.05$; Table 3).

3.4 | Association of CDC42 with DFS and OS in CRC patients

Until the last follow-up, 55 (22.0%) patients had recurrence and 32 (12.8%) patients died. Apart from the classification by quartiles, CDC42 was also divided into low expression (≤50th percentile) and high expression (>50th percentile) to explore the association of CDC42 with prognosis.

A higher CDC42 quartile ($p = 0.002$; Figure 2A) and CDC42 high (vs. low; $p < 0.001$; Figure 2B) were related to worse DFS. In detail, the 5-year DFS rate among patients with CDC42 quartile 1, 2, 3, and 4 were 82.4%, 77.7%, 62.5%, and 55.3%, respectively; meanwhile, 5-year DFS rate among patients with CDC42 low and high was 79.5% and 59.7%, accordingly.

In addition, a higher CDC42 quartile ($p = 0.002$; Figure 3A) and CDC42 high (vs. low; $p = 0.001$; Figure 3B) were also linked to poor OS. Detailly, the 5-year OS rate among patients with CDC42 quartile 1, 2, 3, and 4 was 93.8%, 84.4%, 68.2%, and 67.3%, respectively; meanwhile, the 5-year OS rate among patients with CDC42 low and high was 89.4% and 68.1%, accordingly.

3.5 | Independent factors related to DFS in CRC patients

Multivariate Cox's proportional hazards regression analysis presented that CDC42 quartile 3 (vs. quartile 1) (hazard ratio [HR] = 2.874, $p = 0.024$), CDC42 quartile 4 (vs. quartile 1) (HR = 3.398, $p = 0.007$), ECOG PS score of 1 (vs. 0) (HR = 1.976, $p = 0.017$), poor (vs. well) tumor differentiation (HR = 3.513, $p = 0.042$), T4 (vs. T1 or T2) stage (HR = 60.463, $p < 0.001$), and N2 (vs. N0) stage (HR = 3.221, $p < 0.001$) were independently correlated with declined DFS (Table 4).

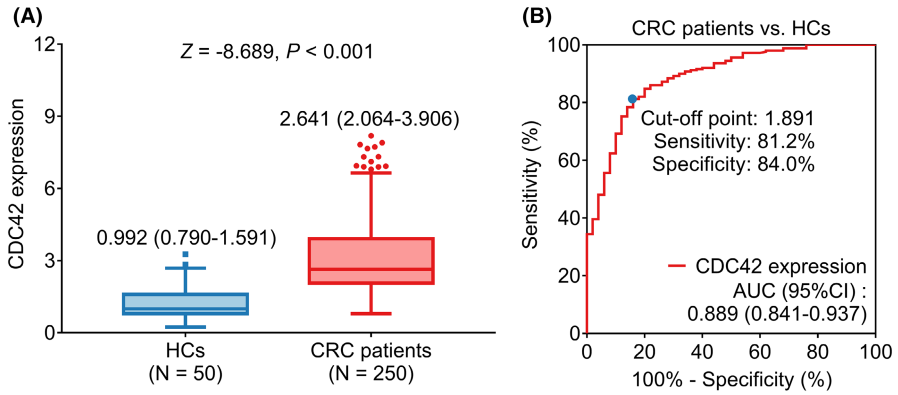

FIGURE 1 CDC42 in CRC patients and HCs. Comparison of CDC42 between CRC patients and HCs (A); the capability of CDC42 in discriminating CRC patients from HCs (B)

TABLE 2 Correlation of CDC42 expression with demographics and comorbidities in CRC patients

Items	CDC42 expression				Statistics (χ^2)	*p* Value
	Quartile 1	Quartile 2	Quartile 3	Quartile 4		
Age, *n* (%)					1.751	0.186
≤60 years	30 (48.4)	25 (39.7)	26 (41.3)	22 (35.5)		
>60 years	32 (51.6)	38 (60.3)	37 (58.7)	40 (64.5)		
Gender, *n* (%)					0.591	0.442
Female	22 (35.5)	26 (41.3)	22 (34.9)	19 (30.6)		
Male	40 (64.5)	37 (58.7)	41 (65.1)	43 (69.4)		
Smoker, *n* (%)					0.956	0.328
No	41 (66.1)	50 (79.4)	43 (68.3)	38 (61.3)		
Yes	21 (33.9)	13 (20.6)	20 (31.7)	24 (38.7)		
Hypertension, *n* (%)					0.835	0.361
No	43 (69.4)	47 (74.6)	41 (65.1)	40 (64.5)		
Yes	19 (30.6)	16 (25.4)	22 (34.9)	22 (35.5)		
Hyperlipidemia, *n* (%)					0.298	0.585
No	51 (82.3)	58 (92.1)	51 (81.0)	51 (82.3)		
Yes	11 (17.7)	5 (7.9)	12 (19.0)	11 (17.7)		
Diabetes, *n* (%)					3.087	0.079
No	54 (87.1)	58 (92.1)	58 (92.1)	47 (75.8)		
Yes	8 (12.9)	5 (7.9)	5 (7.9)	15 (24.2)		

Abbreviations: CDC42, cell division cycle 42; CRC, colorectal cancer.

3.6 | Independent factors related to OS in CRC patients

Multivariate Cox's proportional hazards regression analysis showed that CDC42 quartile 3 (vs. quartile 1) (HR = 7.383, $p = 0.013$), CDC42 quartile 4 (vs. quartile 1) (HR = 7.363, $p = 0.011$), ECOG PS score of 1 (vs. 0) (HR = 2.270, $p = 0.032$), T4 (vs. T1 or T2) stage (HR = 53.084, $p = 0.018$), and N2 (vs. N0) stage (HR = 7.927, $p < 0.001$) were independently correlated with declined OS (Table 5).

In addition, higher ECOG PS score ($P = 0.018$) (Supplementary Figure S1A), higher T stage ($P < 0.001$) (Supplementary Figure S1B), elevated N stage ($p < 0.001$) (Supplementary Figure S1C), and poor tumor differentiation ($p = 0.003$) (Supplementary Figure S1D) were correlated with declined DFS; meanwhile, higher ECOG PS score ($p = 0.034$) (Supplementary Figure S1E), higher T stage ($p < 0.001$) (Supplementary Figure S1F), and elevated N stage ($p < 0.001$) (Supplementary Figure S1G) were associated with decreased OS.

4 | DISCUSSION

It has been reported that CDC42 is highly expressed in CRC tissues.[11,20] While the blood CDC42 in CRC patients is unclear. In the present study, we discovered that blood CDC42 was upregulated in CRC patients compared to HCs, which also had a good ability to distinguish CRC patients from HCs. The possible explanations might be that: (1) CDC42 might suppress the $CD8^+$ T cells activation and promote the immune escape of CRC cells, which could accelerate the tumorigenesis of CRC[10,11]; (2) CDC42 could elevate macrophage recruitment, consequently promoting the pathogenesis of CRC.[21] Thereby, blood CDC42 was increased in CRC patients.

The correlation of CDC42 with clinical features among cancer patients has been paid a lot of attention. For instance, an interesting study has presented that blood CDC42 is not linked to patients' demographic information and comorbidities (such as hypertension, hyperlipidemia, and diabetes), while it is related to elevated lymph node metastasis, higher TNM stage, and rising ECOG PS score among lung cancer patients.[18] However, the data about the relation of blood CDC42 with clinical characteristics among CRC patients are scarce. The present study discovered that blood CDC42 was linked to higher T stage, N stage, TNM stage, abnormal CEA, and adjuvant therapy administration among CRC patients. The potential explanation might be that: (1) CDC42 could suppress $CD8^+$ T cells activation and promote the immune escape, consequently inducing the tumor growth and invasion, which resulted in higher T, N, and TNM stages[10,13,22]; (2) CDC42 might be able to directly accelerate tumor growth and invasion through several pathways, such as vascular endothelial growth factor and membrane-anchored neuropilin-1 signalings, which could lead to elevated T, N, and TNM stages[14–16]; (3) CDC42 could accelerate tumor growth and invasion, as well as correlate with higher T, N, and TNM stage (above-mentioned), which led to the increment of tumor marker (CEA); (4) CDC42 was related to higher TNM stage (above-mentioned); meanwhile, TNM stage could critically affect whether patients would receive adjuvant

TABLE 3 Correlation of CDC42 expression with tumor features, tumor marker and adjuvant treatment information in CRC patients

Items	CDC42 expression				Statistics (χ^2)	*p* Value
	Quartile 1	Quartile 2	Quartile 3	Quartile 4		
Diagnosis, *n* (%)					0.443	0.506
Colon	42 (67.7)	42 (66.7)	38 (60.3)	47 (75.8)		
Rectum	20 (32.3)	21 (33.3)	25 (39.7)	15 (24.2)		
ECOG PS, *n* (%)					1.287	0.257
0	35 (56.5)	36 (57.1)	31 (49.2)	30 (48.4)		
1	27 (43.5)	27 (42.9)	32 (50.8)	32 (51.6)		
Tumor differentiation, *n* (%)					2.669	0.102
Well	15 (24.2)	12 (19.0)	12 (19.0)	7 (11.3)		
Moderate	28 (45.2)	29 (46.1)	30 (47.6)	30 (48.4)		
Poor	19 (30.6)	22 (34.9)	21 (33.4)	25 (40.3)		
Tumor size, *n* (%)					2.605	0.107
<5 cm	45 (72.6)	43 (68.3)	40 (63.5)	37 (59.7)		
≥5 cm	17 (27.4)	20 (31.7)	23 (36.5)	25 (40.3)		
T stage, *n* (%)					15.787	<0.001
T1	3 (4.8)	2 (3.2)	1 (1.6)	0 (0.0)		
T2	11 (17.7)	10 (15.8)	5 (7.9)	1 (1.6)		
T3	48 (77.5)	51 (81.0)	56 (88.9)	59 (95.2)		
T4	0 (0.0)	0 (0.0)	1 (1.6)	2 (3.2)		
N stage, *n* (%)					6.906	0.009
N0	47 (75.8)	38 (60.3)	37 (58.7)	32 (51.6)		
N1	11 (17.7)	17 (27.0)	16 (25.4)	20 (32.3)		
N2	4 (6.5)	8 (12.7)	10 (15.9)	10 (16.1)		
TNM stage, *n* (%)					14.676	<0.001
Stage I	14 (22.6)	12 (19.0)	6 (9.5)	1 (1.6)		
Stage II	33 (53.2)	26 (41.3)	31 (49.2)	31 (50.0)		
Stage III	15 (24.2)	25 (39.7)	26 (41.3)	30 (48.4)		
CEA, *n* (%)					4.080	0.043
Normal	43 (69.4)	38 (60.3)	36 (57.1)	32 (51.6)		
Abnormal	19 (30.6)	25 (39.7)	27 (42.9)	30 (48.4)		
Adjuvant chemotherapy, *n* (%)					9.168	0.002
No	19 (30.6)	18 (28.6)	10 (15.9)	7 (11.3)		
Yes	43 (69.4)	45 (71.4)	53 (84.1)	55 (88.7)		
Adjuvant regimen, *n* (%)					1.561	0.212
Capecitabine	13 (30.2)	10 (22.2)	16 (30.2)	9 (16.4)		
CapeOx	30 (69.8)	35 (77.8)	37 (69.8)	46 (83.6)		

Abbreviations: CapeOX, capecitabine plus oxaliplatin; CDC42, cell division cycle 42; CEA, carcinoembryonic antigen; CRC, colorectal cancer; ECOG PS, Eastern Cooperative Oncology Group Performance Status.

chemotherapy[23]; thus, CDC42 was correlated with adjuvant chemotherapy administration.

The relation of CDC42 with survival profile among cancer patients has been investigated. For instance, blood CDC42 is negatively associated with unsatisfactory DFS and OS among lung cancer patients[18]; moreover, it also has been proposed that the increment of CDC42 in tumor tissue leads to an unfavorable prognosis among ovarian cancer and CRC patients.[16,24] While the correlation of blood CDC42 with survival among CRC patients is unclear. In the present study, we first discovered that higher CDC42 quartiles were correlated with declined DFS and OS; then, CDC42 was also divided into low expression and high expression for analysis, which illustrated that CDC42 high (vs. low) was also linked to decreased DFS and OS; subsequently, multivariate Cox's proportional hazards regression analysis presented that higher CDC42 quartile was independently related to declined DFS and

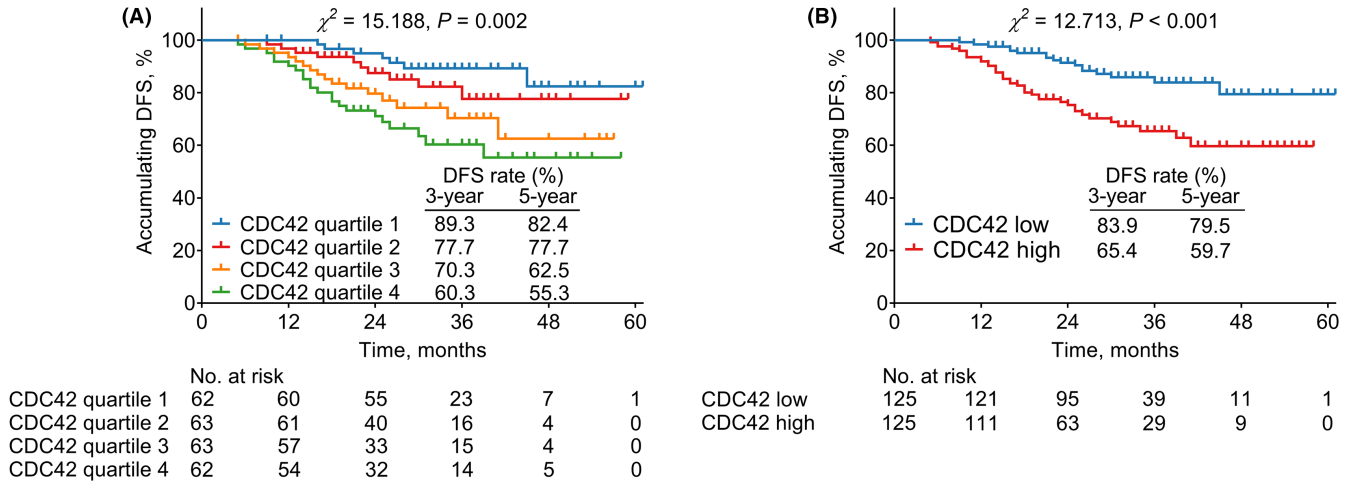

FIGURE 2 Relation of CDC42 with DFS in CRC patients. Comparison of DFS among patients with different CDC42 quartiles (A); comparison of DFS between patients with CDC42 high and patients with CDC42 low (B)

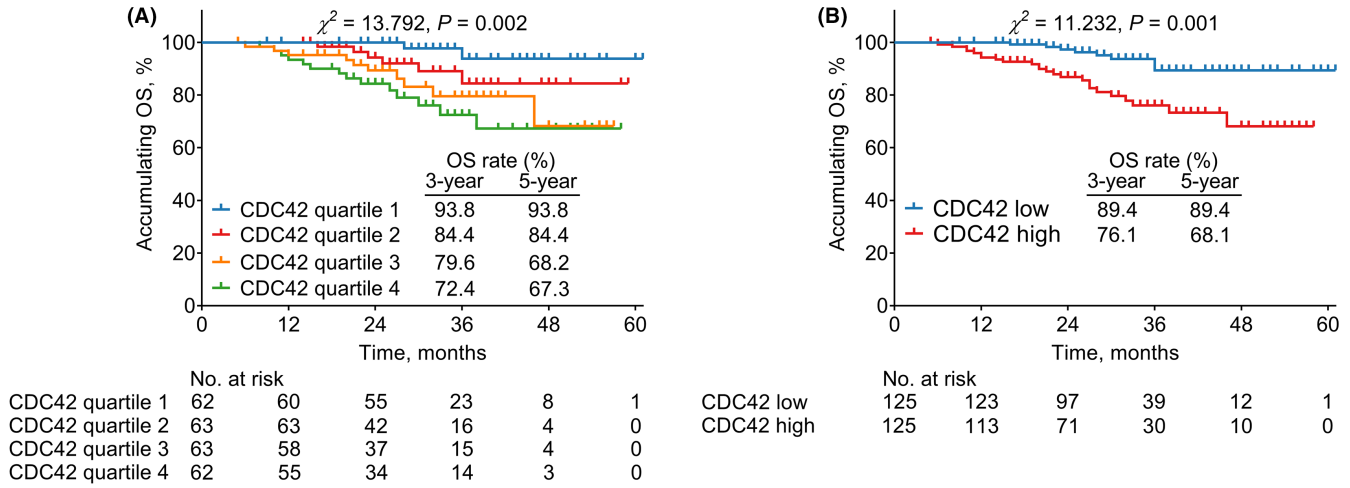

FIGURE 3 Correlation of CDC42 with OS in CRC patients. Comparison of OS among patients with different CDC42 quartiles (A); comparison of OS between patients with CDC42 high and patients with CDC42 low (B)

OS. The above data indicated that blood CDC42 might have the potential to serve as the prognosis biomarker in CRC. The potential explanations might be that (1) blood CDC42 was directly correlated with poor tumor features among CRC patients (abovementioned); thus, CDC42 was indirectly associated with declined DFS and OS. (2) CDC42 could promote the immune escape of tumor cells, which might elevate the risk of tumor recurrence, consequently resulting in unfavorable DFS and OS[10,13]; (3) CDC42 could induce drug resistance through promoting transcription factor SRY-box transcription factor 2, consequently attenuate the efficacy of adjuvant chemotherapy and resulting in poor prognosis among CRC patients.[25] Thereby, blood CDC42 was related to declined DFS and OS among CRC patients. Apart from that, we also discovered that higher ECOG PS score, poor differentiation, and higher T and N stage were also independently predicted poor prognosis among CRC patients, indicating patients with these clinical features should be more attention by clinicians.

The reasons for detecting blood CDC42 among CRC patients were as follows: (1) it was convenient to acquire blood samples with less harm among patients and (2) blood samples could be obtained before surgical resection, which could facilitate the early classification and management of patients. However, the present study exited some limitations: (1) as a single-center study, the generalization of research might be affected by selection bias; (2) CRC patients with distant metastases were excluded in the present study; hence, the clinical role of blood CDC42 in these patients could be explored; (3) the underlying mechanism of CDC42 in the progression of CRC could be discovered in the future; (4) because CDC42 could regulate $CD8^+$ T cells and immune escape, the association of CDC42 with immunotherapy could be investigated among CRC patients later; (5) the modulation of CDC42 in $CD8^+$ T cell activation and immune escape among CRC patients could be investigated in the future; and (6) we only detected the mRNA expression of CDC42 in the current study, the protein expression of CDC42 could be explored in the forthcoming research.

TABLE 4 Independent factors related to DFS by multivariate Cox's proportional hazards regression analysis with forward-stepwise mode

Items	*p* value	HR	95% CI	
			Lower	Upper
CDC42 expression				
Quartile 1	Ref.			
Quartile 2	0.304	1.667	0.629	4.413
Quartile 3	0.024	2.872	1.148	7.185
Quartile 4	0.007	3.398	1.394	8.283
ECOG PS				
0	Ref.			
1	0.017	1.976	1.132	3.451
Tumor differentiation				
Well	Ref.			
Moderate	0.431	1.638	0.479	5.594
Poor	0.042	3.513	1.046	11.799
T stage				
T1 or T2	Ref.			
T3	0.519	1.498	0.439	5.108
T4	<0.001	60.463	6.971	524.414
N stage				
N0	Ref.			
N1	0.352	0.690	0.315	1.509
N2	<0.001	3.221	1.706	6.080

Abbreviations: CDC42, cell division cycle 42; CI, confidence interval; DFS, disease-free survival; ECOG PS, Eastern Cooperative Oncology Group Performance Status; HR, hazard ratio.

TABLE 5 Independent factors related to OS by multivariate Cox's proportional hazards regression analysis with forward-stepwise mode

Items	*p* value	HR	95% CI	
			Lower	Upper
CDC42 expression				
Quartile 1	Ref.			
Quartile 2	0.118	3.624	0.720	18.239
Quartile 3	0.013	7.383	1.530	35.638
Quartile 4	0.011	7.363	1.587	34.163
ECOG PS				
0	Ref.			
1	0.032	2.270	1.071	4.810
T stage				
T1 or T2	Ref.			
T3	0.836	1.248	0.152	10.228
T4	0.018	53.084	1.982	1421.918
N stage				
N0	Ref.			
N1	0.422	1.528	0.543	4.294
N2	<0.001	7.927	3.283	19.143

Abbreviations: CDC42, cell division cycle 42; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group Performance Status; HR, hazard ratio; OS, overall survival.

To be conclusive, circulating CDC42 relates to higher disease risk, T, N, and TNM stage, abnormal tumor marker, and poor prognosis among CRC patients, suggesting that circulating CDC42 may be served as a biomarker to help the early stratification of CRC patients, thus improving their management.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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