Original Research

Ciz1 is a novel predictor of survival in human colon cancer

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Abstract

Cip1-interacting zinc-finger protein1 (Ciz1) is a nuclear matrix protein associated DNA replication factor which has been implicated in breast and lung cancer progression. However, the clinical significance of Ciz1 expression in colon cancer has not been determined. This study aimed to examine Ciz1 expression pattern and its potential as a biomarker of prognosis in colon cancer. Using quantitative PCR, tissue microarray (TMA), and ELISA, we evaluated Ciz1 mRNA and protein levels in tumor tissues from patients with colon cancer and in paired adjacent normal tissues. Ciz1 mRNA expression was significantly upregulated in 22 of 39 paired samples ($P < 0.001$). Immunohistochemistry on TMA-containing samples from 203 colon cancer patients indicated that Ciz1 protein expression was significantly higher in tumor tissues than in adjacent normal tissues (Stuart-Maxwell test, $P < 0.001$). Elevated expression of Ciz1 protein was significantly correlated with T stage ($P < 0.001$), N stage ($P = 0.005$), M stage ($P = 0.021$), and AJCC stage ($P = 0.002$). Multivariate Cox proportion hazard model analysis revealed that Ciz1 expression is an independent prognostic factor for overall time (OS; hazard ratio (HR): 1.76; 95% confidence interval (CI): 1.04–2.98; $P = 0.034$) and disease-free survival (DFS; HR: 2.02; 95% CI: 1.14–3.58; $P = 0.017$) of patients with colon cancer after colectomy. Our data suggested that Ciz1 may be involved in colon cancer progression and could serve as a novel predictor of survival for colon cancer patients.

Keywords: Ciz1, colon cancer, prognosis, tumor progression

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Introduction

Colon cancer is the third most fatal malignancy worldwide, with an increasing incidence in China and other developing countries over the past 20 years.[1,2] Despite the remarkable advances that have been made in colon cancer treatment, its prognosis is still poor, largely as a consequence of tumor recurrence and distant metastasis.[3] Currently, the tumor (T) node (N) metastasis (M) classification developed by the American Joint Committee on Cancer (AJCC) and the International Union for Cancer Control (UICC) remains the most valuable tool to quantify prognosis for individual patient and assist in decision-making for personalized cancer treatment.[4] The restriction of TNM to anatomic information has led clinicians to develop additional prognostic systems.[5] Novel molecular markers of tumor biological behavior will help determine tumor metastatic potential and patients' prognosis.

Deregulated cell-cycle control is one of the fundamental mechanisms of development and progression of colon cancer.[6] Cell cycle-associated proteins function to regulate checkpoint control and cell cycle arrest.[7] Cip1-interacting zinc-finger protein1 (Ciz1) was initially identified as an interacting protein of Cip1, a major regulator of the G1/S cell cycle.[8–10] Later it was characterized as a nuclear matrix associated DNA replication factor mapped to the human chromosome band 9q34.[10–12] The Ciz1 protein was detected in a variety of tissues, including pancreas, brain, intestine, kidney, and placenta.[13–15] It contains two glutamine-rich domains in the N-terminal end, three zinc-finger motifs, and a MH3 domain at the C-terminal end.[10]

Ciz1 plays important roles in DNA synthesis and cell cycle control.[11,14,15] Dysregulation of Ciz1 induces aberrant alteration in proliferation, differentiation, and apoptosis of cancer cells.[14–17] An increase of Ciz1 expression has been observed in a number of human cancerous tissues.[16–19] In a human breast cancer cell line, Ciz1 regulates cell cycle progression by modulating CDK2 activity and the G1-S transition.[18] Ciz1 was estrogen-responsive, and overexpression of Ciz1 enhanced estrogen hypersensitivity of breast cancer cells and induced tumor formation in nude mice.[16] Lukasik *et al.* found that Ciz1 and enhancer of rudimentary homolog (ERH) are coexpressed in Hela cells by overlapping their specific binding site, regulating DNA replication and transcription.[19] Ciz1 also interacts with p53 and DNA-damage-regulated protein PDRG1 to regulate apoptosis and cell cycle.[14] In addition, interaction of Ciz1 with the cytoplasmic scaffold protein SH3BP4 potentially regulates cell proliferation in pancreatic ductal adenocarcinoma.[15] Recent studies showed that aberrant splicing of exon 4 of Ciz1 variants is associated with controls cell proliferation of medulloblastoma and Ewing sarcoma.[11,13,17,20] Therefore, Ciz1 emerged as a novel cancer-related protein.

To date, Ciz1 expression has not been studied in colon cancer. In the present study, we employed a set of TMA including samples from 203 patients with colectomy, and examined Ciz1 expression in colon cancer tissues and adjacent normal colonic mucosa. We also established the correlation between the expression of Ciz1 and clinicopathological parameters of colon cancer. In addition, we investigated whether Ciz1 expression was associated with the risk of disease recurrence and patient survival.

Materials and methods

Tissue specimens

All patient-derived specimens were collected and archived under protocols approved by the Institution Review Boards of Shanghai Jiaotong University and the affiliated Shanghai First People's Hospital. Each patient had signed a written consent form approved by the Institutional Review Board of Shanghai First People's Hospital before surgery. A total of 203 patients who had radical colectomy at Shanghai Jiaotong University Affiliated first People's Hospital between 2001 and 2003 were recruited for this study. None of these patients had received preoperative chemotherapy or radiotherapy. After surgical resection, the grossly visible cancerous and normal tissues (at least 2 cm away from the tumor edge) were snap-frozen in liquid nitrogen and stored at −80°C. The frozen sections of these specimens were stained with hematoxylin and eosin (H & E) and examined by two pathologists. Tumor classification is based on the American Joint Committee on Cancer sixth edition cancer staging system. These patients include 86 men and 117 women, with a median age of 68 years (ranging from 22 to 95 years).

The patients after colectomy were subjected to a yearly colonoscopy and other regular tumor screening tests including computed tomography of chest, abdomen, and pelvis. Patient follow-up was performed in accordance with the National Comprehensive Cancer Network (NCCN) Practice Guidelines in colon cancer. Disease-free survival (DFS) and overall survival (OS) rates were defined as the interval from the date of surgery to the date of clinically proven recurrence/metastasis and the date of death, respectively.[21] The final follow-up date was 29 June 2008, with a median time of 61 months (ranging from 9 to 89 months).

Tissue microarray construction

The paraffin blocks of human colon cancer specimens were collected for TMA construction. These paraffin blocks included 203 matched samples of primary colon tumor mucosa and adjacent normal mucosa as well as 66 samples of matched metastatic lymph nodes. TMA slides were constructed in collaboration with Shanghai Biochip. Two cores were collected from each of paraffin-embedded primary colon cancer or normal tissues using a punch instrument of 2.0 mm diameter. Samples from the same patient were spotted next to each other to ensure homogeneous staining of the TMA sections.

Immunohistochemical analysis

These TMAs were processed for immunohistochemical staining as described previously.[22] Briefly, these TMAs were incubated with a rabbit polyclonal anti-Ciz1 antibody (ab102013, Abcam, Cambridge, MA, USA) with a dilution of 1:800 at 4°C overnight, and washed and incubated with goat anti-rabbit Envision System Plus-HRP (Dako Cytomation, Copenhagen, Denmark) for 30 min at room temperature. These sections were washed and incubated with 3,3′-d iaminobenzidine tetra-hydrochloride (DAB) for 1 min, followed by counterstaining with hematoxylin, dehydration, and mounting with neutral gums. The negative control was prepared using the same protocol but without primary antibody incubation.

After staining, the tissue sections were reviewed and scored by two double-blinded pathologists. The evaluation of staining intensity was graded as follows: 0 (negative), 1 (weak), 2 (moderate), or 3 (strong). The staining area was scored using the following scale: 0 (0%), 1 (<10%), 2 (10–50%), or 3 (>50%). These scores were combined to produce the final score: 0–2, negative expression; 3–4, weak expression; or 5–6, strong expression. Both weak and strong expressions were considered as positive expression. When a discrepancy in the assessment was encountered, the slides were re-examined by both pathologists under a multi-head microscope to obtain an agreement.

RNA extraction and real-time PCR

Total RNA from frozen tissues was extracted with an ALLPrep DNA/RNA kit (Qiagen, Hilden, Germany) according to the manufacture's instructions. A 2 μg aliquot of total RNA from each sample was used for synthesis of cDNA using a High-Capacity cDNA Reverse Transcription Kits (Applied Biosystems Inc, Foster City, CA, USA). The Ciz1 mRNA expression levels in colon cancerous tissues and adjacent normal tissues were examined on a Mastercycler ep realplex Real-Time Quantitative (qPCR)

system (Eppendorf AG, Hamburg, Germany) with a SYBR Green kit (MBI Fermentas, Hamilton, Canada). Primers for quantitative RT-PCR were as follows: Ciz1: sense 5′-taccagcactccctctaagca-3′, antisense 5′-ctcctcatcctctgtctccag-3′; glyceraldehyde 3-phosphate dehydrogenase: sense 5′-TGACTTCAACAGCGACACCCA-3′, antisense 5′-CACCCTGTTGCTGTAGCCAAA-3′. Each reaction was repeated in triplicate, and the experiments were repeated at least twice to confirm reproducibility.

ELISA

The tissue protein extracts were prepared as previously described.[22] The cellular protein was extracted from tumor and adjacent normal tissue using the Whole Protein Extraction Kit (Fermentas), and the concentration was determined using the BCA protein assay kit (PIERCE, Rockford, IL, USA). Human Ciz1 ELISA was performed using an ELISA procedure developed in house. Briefly, primary anti-Ciz1 antibody (ab102013, Abcam, Cambridge, MA, USA) was used to coat a 96-well microplate overnight at 4°C. Blocking was performed with PBS containing 1% BSA at 37°C for 1 h. After washing, the samples were diluted to a final concentration of 10 μg/ml and transferred to the wells. Following a 2-h incubation, plates were washed and incubated with biotinalyated secondary antibody for 1 h, then washed again and incubated with HRP-avidin for 1 h. After washing, TMB substrate was added to each well for 20 min with protection from light and stop solution was added into the wells after incubation. The optical density was measured at a wavelength of 450 nm with a wavelength correction of 540 nm using automated optical densitometry. The cell lysate from Hela cells was used as positive control. Purified recombinant Ciz1 (Abnova, Taiwan) was tested at concentrations ranging from 156 pg/mL to 10 ng/mL to generate a standard curve. Each sample was run in triplicate, and the mean value was used for analysis.

Statistical analysis

Paired *t*-tests were used to analyze the level of Ciz1 mRNA expression. Chi-square tests or Fisher's exact test was used to examine the associations between clinicopathological parameters and Ciz1 protein expression. Survival curves were plotted by Kaplan–Meier curves with log-rank tests. In addition, univariate and multivariate Cox-regression analyses were used to validate the independent factors for survival and lymph node metastasis. All the above statistical analyses were performed using SPSS19.0 statistical software (SPSS Inc, IBM, USA) with a significance level of 0.05. Stuart-Maxwell test was used to analyze the protein expression level of Ciz1 in tumor and adjacent normal tissues from the TMAs.

Results

Upregulation of Ciz1 mRNA expression in colon cancer tissues

Real-time PCR analysis of Ciz1 mRNA expression was performed on 39 paired clinical samples of colon cancer tissues and adjacent normal tissues. The expression of Ciz1 mRNA in cancerous tissues was significantly higher than that in normal tissues (Figure 1(a); $^*P < 0.001$). The Ciz1 mRNA level of 22 (56.4%) cases of colon cancer tissues exhibited at least a 2-fold increase compared with normal colon tissues (Figure 1b).

Association of Ciz1 protein expression with clinicopathological parameters in colon cancer

We examined Ciz1 protein expression on the TMAs of 203 paraffin-embedded colon cancer tissues and 66 cases of LNM by immunohistochemistry. Figure 2 represented the immunostaining profiles of Ciz1 in colon cancer. Ciz1 staining was weak or negative on normal colon tissues (Figure 2a), but strong positive staining was observed in the cytoplasm of the tumor cells in both well (Figure 2b) and poorly differentiated colon cancer tissues (Figure 2c). In a total of 203 cases, we found that 94 (46.3%) normal colonic mucosa samples showed weak Ciz1 expression and the remaining 109 (53.7%) samples were negative; 72 (35.5%) of the tumor tissue samples showed strong positive staining, 55 (27.1%) samples showed weak staining, and 76 (37.4%) samples showed negative staining. Stuart-Maxwell test indicated that Ciz1 protein expression is significantly higher in tumor tissues than in adjacent normal tissues ($P < 0.001$, Table 1). Ciz1 protein expression was also examined by tissue ELISA using samples from three patients with colon cancer. The sensitivity of the ELISA was 10 pg/mL with intra-assay and inter-assay coefficients of variation of 4.5% and 7.5%, respectively. Ciz1 protein level was found significantly higher in tumor tissues than in adjacent normal tissues of all the three patients (Figure 3).

Chi-square test or Fisher's exact test showed that the expression level of Ciz1 protein was significantly correlated with T stage ($P = 0.001$), N stage ($P = 0.005$), M stage ($P = 0.021$), and AJCC stage ($P = 0.002$) (Table 2). No correlation was found between Ciz1 protein expression and age ($P = 0.068$), gender ($P = 0.753$), tumor location ($P = 0.126$), vascular invasion ($P = 0.365$), or tumor histological differentiation ($P = 0.217$) (Table 2).

Association between Ciz1 expression and colon cancer patient survival

We analyzed the possible association between Ciz1 expression and patient survival. In the clinical follow-up data, eight patients with stage IV disease who underwent non-curative surgery were excluded, and the remaining 195 patients who underwent colectomy were included for the survival analysis. Patients with negative Ciz1 protein expression had an improved five-year OS and DFS than those who had positive Ciz1 protein expression ($P < 0.001$, respectively) (Figure 4). Kaplan–Meier curves showed that the recurrence was significantly elevated in patients with positive Ciz1 expression (Figure 4b). In addition, COX regression analysis revealed factors that affect colon cancer patient survival (Table 3). Based on the univariate analysis, T_2, T_3, N_1, N_2, M category, AJCC III & IV, vascular invasion, differentiation, and Ciz1 expression were significantly associated with both OS and DFS. The follow-up

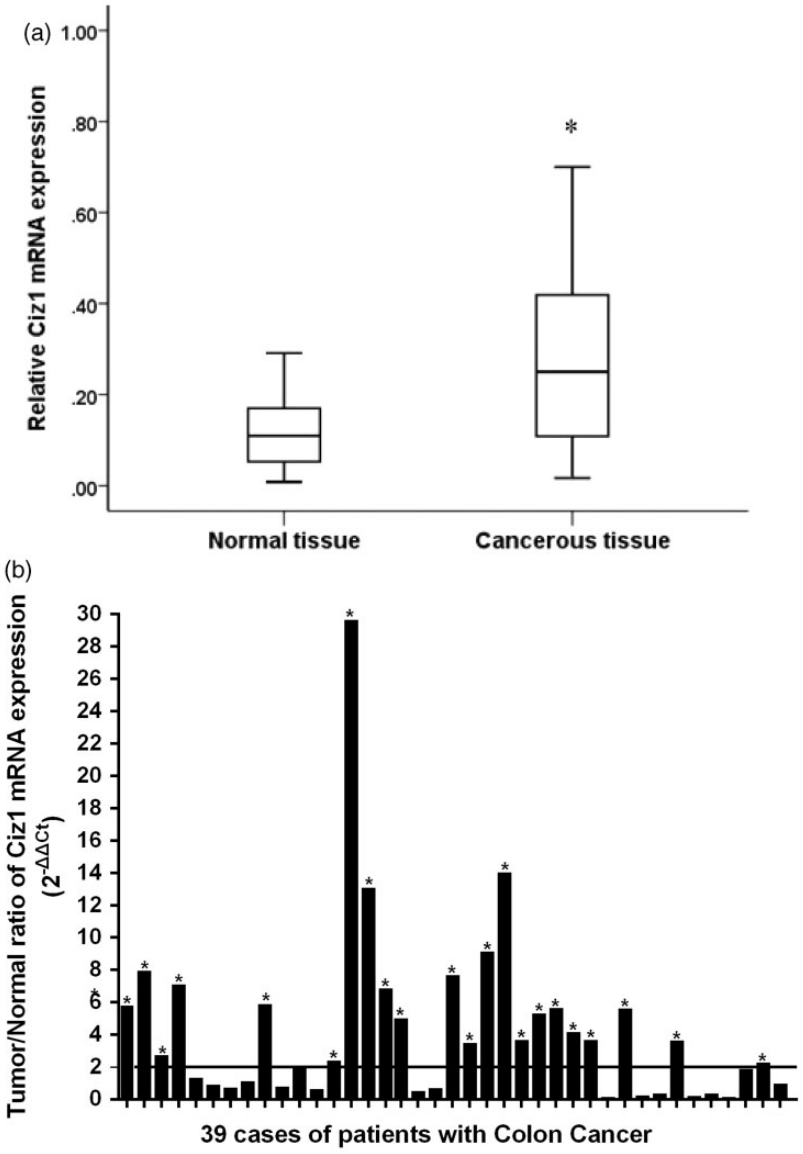

Figure 1 Ciz1 mRNA expression in colon cancer tissues. (a): Real-time PCR analysis relative Ciz1 mRNA expression level in 39 paired of colon tumor samples and adjacent normal mucosa. The relative Ciz1 mRNA level was normalized by GAPDH expression. Ciz1 mRNA expression in normal and cancerous tissues was significantly different (*$P < 0.001$). (b): Each bar represents the tumor/normal tissue ratio of Ciz1 mRNA in paired colon tumor and adjacent normal mucosa from each patient (*represents the tumor/normal ratio of Ciz1 mRNA expression>2-fold)

multivariate analysis also found a strong association of Ciz1 protein expression with OS and DFS of colon cancer patients (HR, 95% (CI): OS: 1.76 (1.04–2.98), $P = 0.034$; DFS: 2.02 (1.14–3.58), $P = 0.017$), suggesting Ciz1 as an independent prognostic factor. Collectively, our data suggested that Ciz1 might represent as a useful biomarker for the prognosis of colon cancer patients after colectomy.

Discussion

To the best of our knowledge, this is the first report demonstrating the association of Ciz1 up-regulation with colon cancer progression. Our data also indicated that patients with Ciz1-positive staining had significantly shorter overall and DFS. Together, these results strongly suggested that Ciz1 could serve as a prognostic factor for patients with colon cancer.

The involvement of Ciz1 in cancer was first reported by a study in which Ciz1 cDNA was isolated from a medulloblastoma library as an agent associated with solid tumor formation in nude mice.[13] From *in vitro* studies, Ciz1 mRNA levels were elevated in lung cancer and Ewing tumors cell lines.[16,17] Increase of Ciz1 mRNA and protein was also found in brain tumors and breast cancer tissues.[13,15,16] In the present study, we found that Ciz1 mRNA levels were significantly higher in colonic cancerous tissues compared with the adjacent normal tissues. Further validation by immunohistochemistry indicated that positive Ciz1 staining was observed on a majority of colonic cancerous tissues, suggesting that Ciz1 might play a role in the development and progression of colon cancer. Ciz1 regulates cell proliferation in cancer cells, possibly by its function on modulating cell cycle.[16,18,19] A recent study

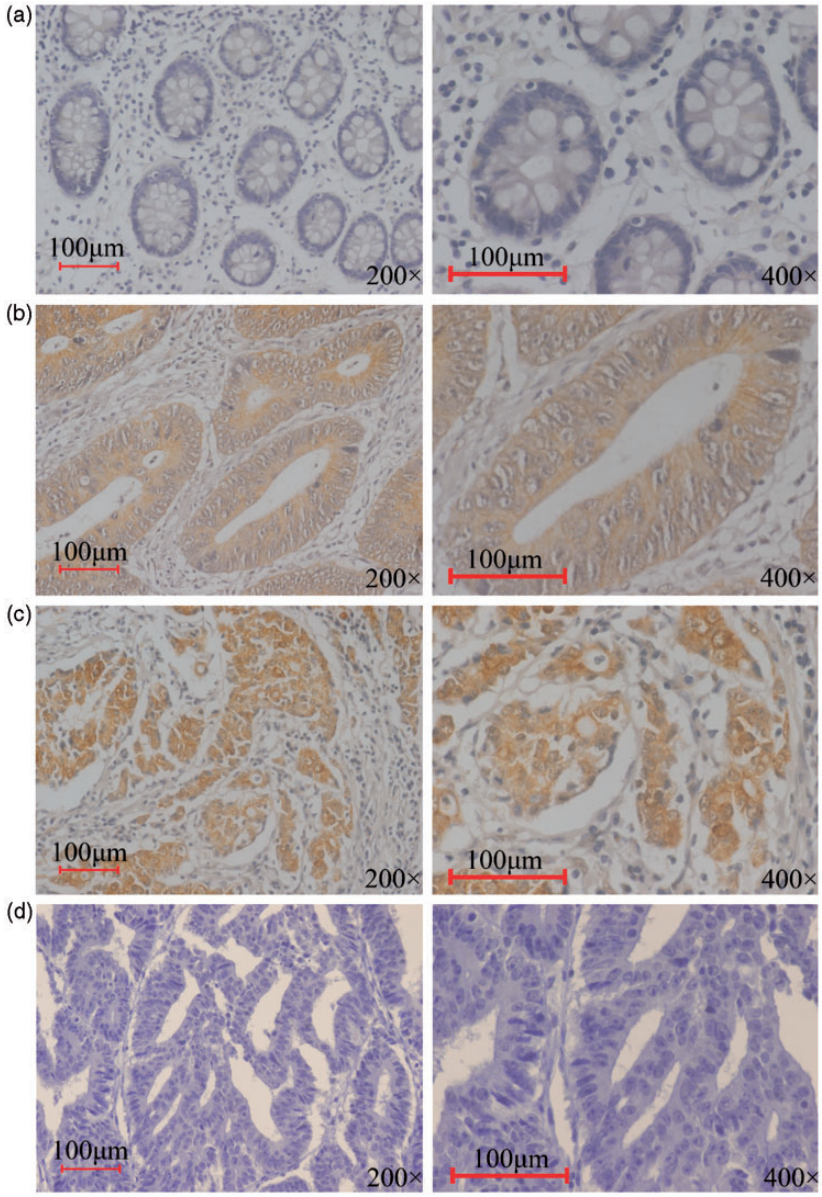

Figure 2 Immunostaining of Ciz1 in colon cancer tissues. (Original magnification: left 200×, right 400×). Ciz1 protein was negative in normal colonic mucosa (a), but positive staining was observed in cytoplasm of the tumor cells in well (b) and poorly differentiated colon cancer tissues (c). Negative controls were stained by skipping primary antibody incubation (d). Bar = 100 μm. (A color version of this figure is available in the online journal.)

Table 1 Ciz1 protein expression in normal and tumor tissues

Colon cancer patients ($n = 203$)	Normal tissues			
	Negative	Weak	Strong	Total *n* (%)
Tumor tissues				
Negative	34 (16.7)[a]	42 (20.7)	0 (0)	76 (37.4)
Weak	40 (19.7)	15 (7.4)	0 (0)	55 (27.1)
Strong	35 (17.2)	37 (18.2)	0 (0)	72 (35.5)
Total *n* (%)	109 (53.7)	94 (46.3)	0 (0)	203 (100)

[a]*n* (%). Stuart-Maxwell test statistic: 72.04, df = 2, $P < 0.001$.

showed that RNAi-mediated selective depletion of variant Ciz1 is sufficient to restrain lung tumor cell proliferation.[23] Therefore, Ciz1 may emerge as a novel target for anti-cancer therapy.

Several proteins associated with colon cancer progression have been identified.[24,25–27] Using the same set of TMAs, a strong association was found between overexpression of IMP3 and AJCC stage, pT, pN, distant metastasis, and ki-67.[24] The current study also revealed that Ciz1 protein is significantly associated with AJCC stage, pT, pN, and distant metastasis, suggesting its potential as a new marker for colon cancer progression. Neither IMP3 nor Ciz1 correlates with age, gender, tumor location, and differentiation. Both IMP3 and Ciz1 could serve as a novel predictor to assess the risk of metastasis and recurrence. Therefore, it is worth to explore whether IMP3 and Ciz1 are regulated by a similar mechanism in colon cancer. Interestingly, our results indicated that Ciz1 expression was not associated with vascular invasion, thus its function is restricted in tumor cells and not directly involved in promoting

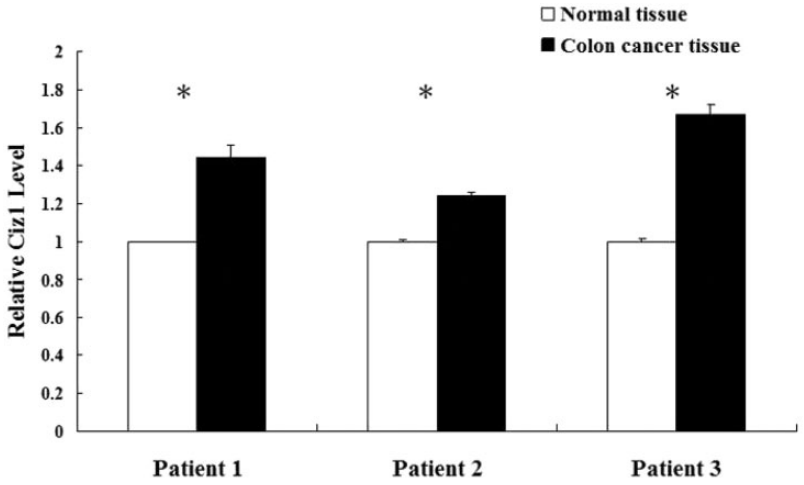

Figure 3 ELISA analysis of Ciz1 protein level in tissue samples from three colon cancer patients. Ciz1 protein expression is significantly higher in tumor tissues than in adjacent normal tissues ($n = 3$; $^*P < 0.01$)

Table 2 Association between Ciz1 protein expression and clinicopathological parameters in colon cancer

	Ciz1 expression *n* (%)			
Clinicopathological variables	**Negative ($n = 76$)**	**Weak ($n = 55$)**	**Strong ($n = 72$)**	***P* value**
Age(years)				0.068
22–45	4 (28.6)	4 (28.6)	6 (42.9)	
46–70	31 (31.0)	35 (35.0)	34 (34.0)	
71–95	41 (46.1)	16 (18.0)	32 (36.0)	
Gender				0.753
Male	34 (39.5)	21 (24.4)	31 (36.0)	
Female	42 (35.9)	34 (29.1)	41 (35.0)	
Tumor location				0.126
Right	30 (35.7)	26 (31.0)	28 (33.3)	
Transverse	4 (21.1)	3 (15.8)	12 (63.2)	
Left	42 (42.0)	26 (26.0)	32 (32.0)	
T stage				0.001
T^1	5 (62.5)	3 (37.5)	0 (0)	
T^2	16 (69.6)	3 (13.0)	4 (17.4)	
T^3	31 (40.8)	18 (23.7)	27 (35.5)	
T^4	24 (25.0)	31 (32.3)	41 (42.7)	
N stage				0.005
N^0	42 (38.9)	36 (33.3)	30 (27.8)	
N^1	21 (34.4)	17 (27.9)	23 (37.7)	
N^2	13 (38.2)	2 (5.9)	19 (55.9)	
M stage				0.021
M^0	74 (40.0)	50 (27.0)	61 (33.0)	
M^1	2 (11.1)	5 (27.8)	11 (61.1)	
AJCC stage				0.002
I	15 (62.5)	5 (20.8)	4 (16.7)	
II	33 (40.7)	15 (18.5)	33 (40.7)	
III	26 (32.5)	30 (37.5)	24 (30.0)	
IV	2 (11.1)	5 (27.8)	11 (61.1)	
Vascular invasion				0.365
No	73 (38.6)	51 (27.0)	65 (34.4)	
Yes	3 (21.4)	4 (28.6)	7 (50.0)	
Differentiation				0.217
Well	44 (44.4)	26 (26.3)	29 (29.3)	
Moderate	25 (33.8)	20 (27.0)	29 (39.2)	
Poor	7 (23.3)	9 (30.0)	14 (46.7)	

Note: *P* value evaluated by Chi-square (χ^2) test or Fisher's exact test.

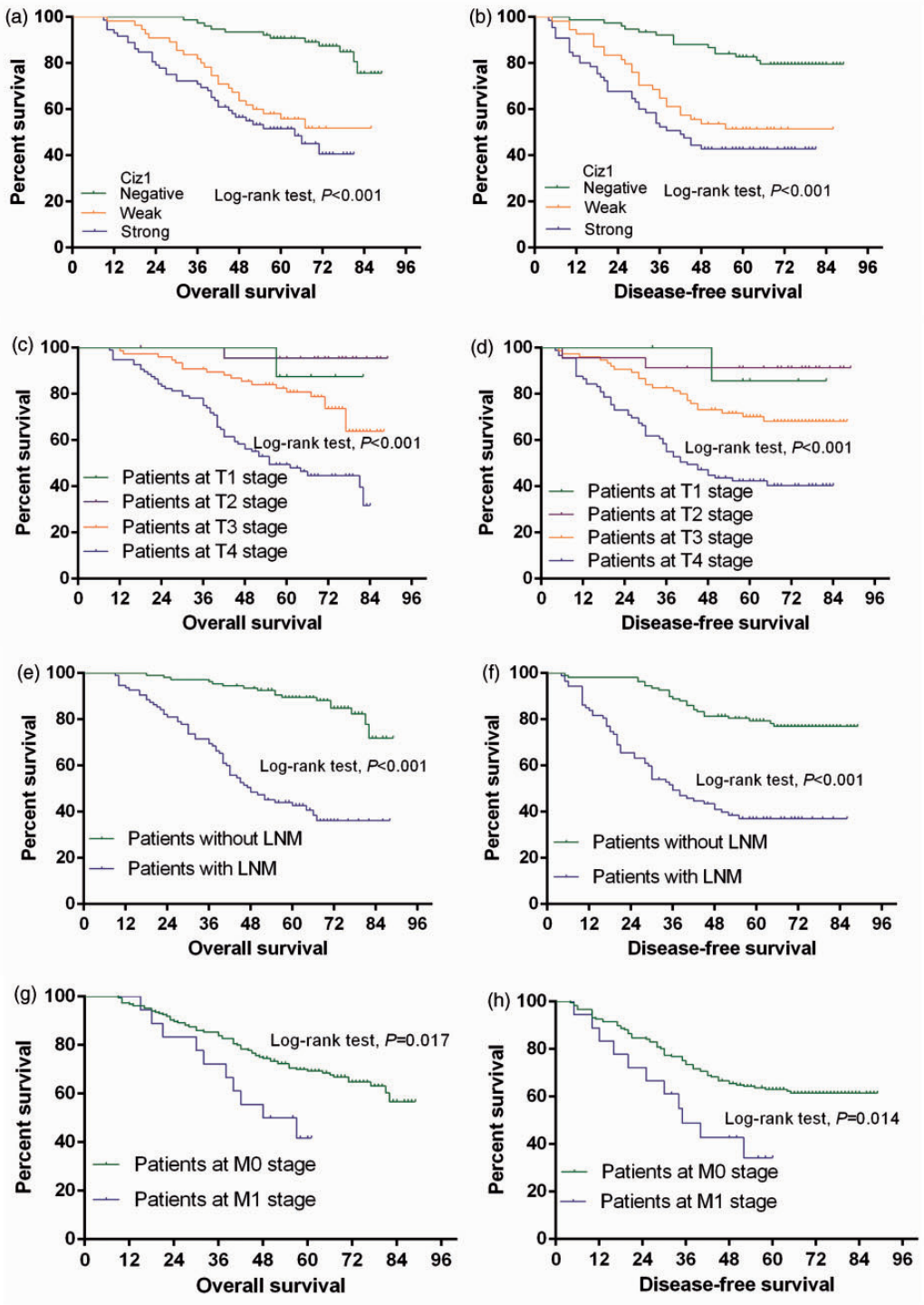

Figure 4 Kaplan–Meier plots of overall survival (OS) and disease-free survival (DFS) of patients with colon cancer who underwent colectomy on the basis of the immunohistochemical Ciz1 expression (a, OS: $P < 0.001$); b, DFS: $P < 0.001$), T stage (c, OS: $P < 0.001$; d, DFS: $P < 0.001$), LNM (e, OS: $P < 0.001$; f, DFS: $P < 0.001$) and M stage (g, OS: $P = 0.017$; h, DFS: $P = 0.014$). (A color version of this figure is available in the online journal.)

angiogenesis. Though Ciz1 is a nuclear matrix protein, we found robust staining of Ciz1 in the cytoplasm of colon tumor cells. In other types of cancers, Ciz1 was also detected in cytoplasm and blood plasma.[10,18,23] Recently, Higgins *et al*. identified a variant Ciz1 in plasma as a quantifiable circulating biomarker for early stage lung cancer.[23] Despite the uncertainty whether the same variant Ciz1 can be detected in blood plasma of colon cancer patients, it opened a new venue to explore the potential of Ciz1 as a biomarker to identify the stages of tumor progression.

Colon cancer is a heterogenous disease. Patients with the same AJCC stage, histopathological features, and treatment strategy might have different clinical outcomes.[28] Molecular biomarkers related to tumor recurrence and

Table 3 Univariate and multivariate Cox proportion hazard models for OS and DFS

	Overall survival				Disease-free survival			
	Univariate		Multivariate		Univariate		Multivariate	
Variables	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
Age(years)								
22–45	–				–			
46–70	0.30 (0.04–2.15)	0.232			0.48 (0.08–2.89)	0.425		
71–95	0.95 (0.22–4.09)	0.944			1.26 (0.41–5.34)	0.756		
Gender								
Male	0.74 (0.46–1.20)	0.223			–			
Female	–				0.88 (0.56–1.38)	0.581		
Tumor location (cm)								
Right	–				–			
Transverse	0.80 (0.33–1.93)	0.618			0.83 (0.34–1.98)	0.669		
Left	1.04 (0.64–1.69)	0.862			1.12 (0.70–1.79)	0.631		
T stage								
T_1	0.36 (0.09–1.46)	0.152	0.33 (0.07–1.65)	0.170	0.34 (0.08–1.39)	0.132	0.41 (0.10–1.78)	0.236
T_2	0.11 (0.03–0.44)	0.002	0.20 (0.02–2.15)	0.180	0.16 (0.05–0.52)	0.002	0.27 (0.06–1.15)	0.075
T_3	0.34 (0.20–0.58)	<0.001	0.35 (0.20–0.62)	<0.001	0.42 (0.26–0.70)	0.001	0.42 (0.25–0.70)	0.001
T_4	–		–		–		–	
N stage								
N_0	–		–		–		–	
N_1	3.74 (1.96–7.12)	<0.001	3.45 (1.66–6.83)	<0.001	2.73 (1.57–4.73)	<0.001	2.06 (1.46–3.58)	0.019
N_2	15.02 (7.85–28.75)	<0.001	9.67 (4.89–19.12)	<0.001	10.22 (5.78–18.08)	<0.001	7.64 (3.78–15.46)	<0.001
M stage								
M_0	–		–		–		–	
M_1	14.74 (8.15–26.67)	<0.001	6.32 (1.38–28.98)	<0.001	9.93 (4.91–20.07)	<0.001	7.16 (3.87–13.24)	<0.001
AJCC stage								
I	–				–			
II	2.08 (0.47–9.21)	0.336			2.07 (0.61–6.96)	0.241		
III	9.51 (2.29–39.47)	0.002			6.69 (2.07–21.58)	0.001		
IV	72.12 (16.17–321.61)	<0.001			37.18 (9.95–138.97)	<0.001		
Vascular invasion								
No	–				–			
Yes	4.68 (2.55–8.59)	<0.001			4.12 (2.16–7.86)	<0.001		
Differentiation								
Well	–				–			
Moderate	2.37 (1.34–4.18)	0.003			2.26 (1.35–3.79)	0.002		
Poor	7.50 (4.11–13.68)	<0.001			4.87 (2.64–8.97)	<0.001		
Ciz1								
Negative	–		–		–		–	
Weak/strong	2.13 (1.26–3.59)	0.004	1.76 (1.04–2.98)	0.034	2.24 (1.34–3.72)	0.002	2.02 (1.14–3.58)	0.017

Note: $P < 0.05$ indicates that 95% CI of HR did not include 1.

prognosis of colon cancer, such as Hoxb7, OPG, KRAS, and BRAF, have been investigated.[25–27] However, to date, no valid prognostic biomarkers for colon cancer have been established.[29,30] In the current study, we found that patients with Ciz1-positive staining have an increased risk of tumor recurrence and shorter survival time. In addition, Ciz1 expression levels were much higher in tumors with LNM than that without LNM. Statistical analysis revealed Ciz1 as an independent factor associated with OS and DFS, and it is not related to age, sex, tumor location, or vascular invasion. These results pointed toward the possible prognostic value of Ciz1 in colon cancer. However, our study is limited by the small number of patients with relatively short follow-up time. The prognostic value still needs to be validated in a larger, prospective, controlled clinical study.

In conclusion, Ciz1 expression is upregulated in human colon cancer cells, and its expression level is associated with multiple clinicalpatholgoical factors and survival in patients with colon cancer after colectomy. Ciz1 may be a novel predictor of survival in human colon cancer.

Further studies are necessary to explore the prognostic value of Ciz1 and to investigate molecular mechanisms underlying Ciz1 function in colon cancer progression.

Author contributions: All authors participated in the design, interpretation of the studies, analysis of the data, and review of the manuscript. DQW and KW contributed equally to this work.

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