Synergistic role of Cul1 and c-Myc: Prognostic and predictive biomarkers in colorectal cancer

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Abstract. Colorectal cancer (CRC) is one of the most common malignant tumors, and its high rates of recurrence and metastasis are the important causes of treatment failure in CRC. Therefore, the development of valuable molecular markers to accurately predict the prognosis of CRC patients is vital. In the present study, we determined the expression of Cullin1 (Cul1) and c-Myc in a CRC tissue microarray containing 470 cancer and corresponding normal tissues by immunohistochemistry. We found that Cul1 and c-Myc expression was significantly upregulated in the CRC cancer tissues compared with that noted in the adjacent non-cancer tissues. High Cul1 expression in cancer tissues was associated with depth of invasion (P=0.005), lymph node metastasis (P=0.001) and TNM stage (P=0.015). High c-Myc expression in cancer tissues was significantly positively association with age (P=0.004), depth of invasion (P<0.001), lymph node metastasis (P<0.001) and TNM stage (P<0.001). Multivariate Cox regression analysis revealed that Cul1 or c-Myc expression was an independent and unfavorable prognostic factor for CRC patients [hazard ratio (HR), 0.749, 95% confidence interval (CI), 0.563-0.996, P<0.05; and HR, 0.384, 95% CI, 0.257-0.472, P<0.001, respectively]. Furthermore, Cul1 and c-Myc exhibited synergistic potential for the prediction of CRC prognosis, and the patients with low expression of both Cul1 and c-Myc had a favorable survival outcome (P<0.001).

Introduction

Colorectal cancer (CRC) is a cause of high morbidity and mortality worldwide (1). Despite the use of multi-model treatment strategies, including surgery, perioperative chemotherapy, radiotherapy and targeted therapy, a subset of patients commonly develop local recurrence and metachronous metastasis after resection of the primary tumor (2,3). Therefore, the molecular and cellular processes involved in CRC metastasis are urgently needed to be detected in order to develop reliable biomarkers to predict poor patient outcome.

A major pathway controlling protein degradation is the ubiquitin-proteasome system (4). The attachment of ubiquitin to target proteins is mediated by at least 3 enzymes: an ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2) and a ubiquitin ligase (E3) (5). Cullins are a family of hydrophobic proteins providing a scaffold for ubiquitin ligase E3. Cullin1 (Cul1) is the most representative member of the Cullin protein family, which degrades many proteins by mediating ubiquitination of proteins involved in cell cycle progression, signal transduction and transcription (6-8). Cul1 regulates cell proliferation, cell cycle, migration, invasion, metastasis and is associated with the patient prognosis in gastric cancer and CRC (9,10). Recently, other investigators have reported that Cul1 expression is associated with poor prognosis in melanoma, lung and breast cancer (11-13).

The protoncogene c-Myc is a member of the Myc family protein (14), which is involved in many biological processes such as cell cycle, cell differentiation and protein synthesis (15,16). Overexpression of c-Myc in CRC is a deterioration index (17). The c-Myc gene is known to regulate both oncogenes and tumor-suppressor genes and therefore play an important role in the occurrence and development of cancers. c-Myc directly governs cell mass and progression through critical cell cycle transitions by promoting G_1 exit, and the regulation in part via Cul1-dependent ubiquitination and degradation of the CDK inhibitor, $p27^{kip1}$ (18).

Herein, we aimed to elucidate the expression patterns of Cul1 and c-Myc in a CRC patient cohort, and to examine the possibility of Cul1 or c-Myc alone or in combination as a prognostic and predictive biomarker.

Materials and methods

Patient specimens and tissue samples. The present study was approved by the Institutional Review Board of Yixing

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Abbreviations: Cul1, Cullin1; CRC, colorectal cancer

Key words: Cul1, c-Myc, colorectal cancer, prognosis, marker

Hospital prior to the study. All subjects provided written informed consent and were assured of their anonymity and the confidentiality of the data obtained. The 10-paired fresh samples were frozen in liquid nitrogen immediately after surgical removal and maintained at -80˚C until use for western blot analysis.

The cohort TMA consisting of 470 CRC surgical cases was obtained from Yixing People's Hospital, Yixing City, in the South of Jiangsu Province during January, 2006 and December, 2010. The patients were followed up at least for 5 years. Overall survival (OS) was the primary endpoint of this analysis, and survival time was calculated from the date of surgery to the date of death or to the last follow-up. The patient clinicopathologic information including age at diagnosis, sex, differentiation stage, depth of invasion, lymph node metastasis, tumor-node-metastasis (TNM) stage and tumor diameter was collected. The median age of the patients at tumor resection was 63 years; 281 (59.8%) were male and 189 (40.2%) were female cases (Table I). TMA was constructed by taking tissue portions in the identified and labeled area from the tumor samples which were fixed in formalin and embedded in paraffin.

All procedures involving human participants were in accordance with the ethical standards of the Institutional and/or National Research Committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Western blotting. Western blot analyses were performed as previously described (19). The rabbit anti-Cul1 (1:1,000; Epitomics, Burlingame, CA, USA), anti-c-Myc (1:1,000; Cell Signaling Technology, Inc., Danvers, MA, USA), and monoclonal mouse anti-β-actin antibody (1:2,000; Beyotime Biotechnology, Nantong, China) were used as the primary antibodies. The intensity of the protein bands was analyzed using ImageJ software (version 1.44; Wayne Rasband National Institutes of Health, Bethesda, MD, USA), after normalization to the corresponding β-actin level.

Construction of the tissue microarray (TMA) and immunohistochemistry. Paraffin-embedded archived tissue material of tumor and adjacent normal tissues was used for TMA construction. The CRC TMA included 940 cores. Each sample was punched to a 1.5-mm diameter. The standard protocol used for the immunostaining was provided in a previous study (19). The monoclonal rabbit anti-Cul1 (1:200; Epitomics) and anti-c-Myc (1:200; Cell Signaling Technology, Inc.) were used for primary antibody incubation at 4˚C overnight. The omission of the primary antibody served as the negative control. The staining scores of the tissues controlled in each microarray slide were pre-evaluated as a quality control of the immunostaining.

Evaluation of immunostaining. At first, staining of Cul1 or c-Myc in the tissues was independently scored by two pathologists blinded to the clinical data, by applying a semi-quantitative immunoreactivity score (IRS) in the training cohort. The scoring criteria for IRS were reported elsewhere (9,10,13). The intensity of immunostaining is shown in Figs. 1 and 2. The concordance for IRS staining score of Cul1 between the two pathologists was 423 in 470 tumors (90%), and the discrepancies were resolved by consensus using a multihead microscope. The optimum cut-off value of IRS was obtained by receiver operator characteristic (ROC) analysis; the area under the curve (AUC) at different cut-off values for Cul1 IRS for 1, 3 and 5 years of OS time was calculated. The optimum value of cut-off points for Cul1 IRS was shown to be 4 since it had the best predictive value for survival (Fig. 3). Under these conditions, samples with IRS 0-3 and IRS 4-12 were classified as low or high expression of Cul1, respectively. By use of the same method, the optimum value of cut-off points of c-Myc IRS was shown to be 3 (Fig. 4), and samples with IRS 0-2 and IRS 3-12 were classified as low or high expression of c-Myc, respectively.

Table I. CRC patient clinicopathological data.

Variables	n	%
All patients	470	
Age (years)		
≤65	267	56.8
>65	203	43.2
Sex		
Male	281	59.8
Female	189	40.2
Pathological classification[a]		
I	5	1.1
II	423	91.2
III	36	7.7
Depth of invasion[a]		
T1	9	2.0
T2	94	20.2
T3	347	74.6
T4	15	3.2
Lymph node metastasis[a]		
N0	276	59.2
N1	126	27.0
N2	64	13.8
TNM stage[a]		
I	88	18.9
II	179	38.6
III	180	38.8
IV	17	3.7
Tumor diameter (cm)[a]		
≤5	378	80.6
>5	91	19.4
Distant metastasis		
M0	451	95.9
M1	19	4.1

[a]For some patients, data regarding these clinical pathological parameters were not available. CRC, colorectal cancer; TNM, tumor-node-metastasis.

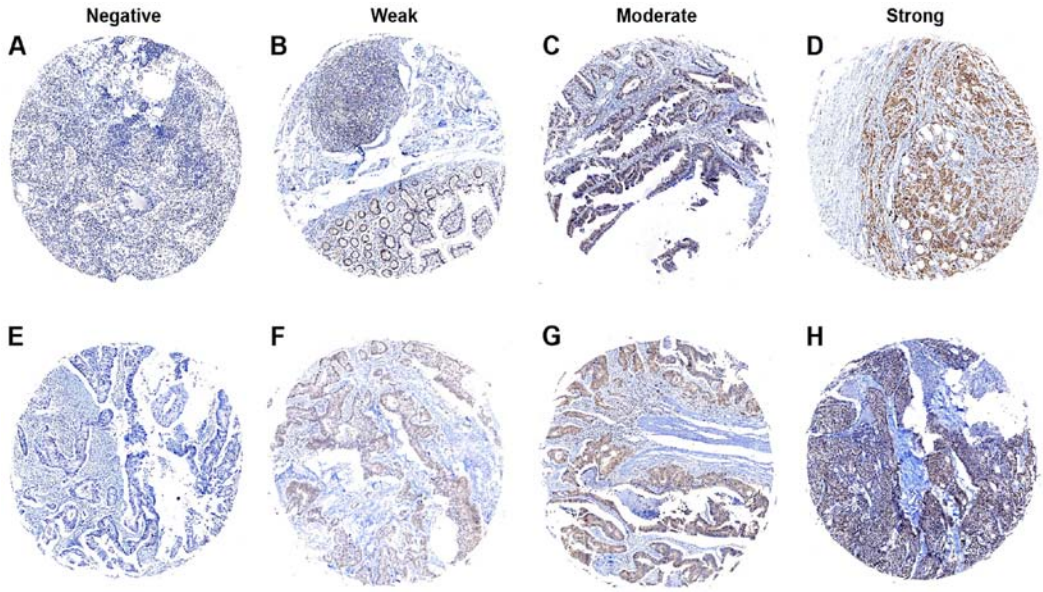

Figure 1. Representative images of Cul1 immunohistochemical staining in CRC cancer and adjacent normal tissues. (A-D) Adjacent normal tissue. (E-H) Cancer tissue. (A and E) Negative staining. (B and F) Weak staining. (C and G) Moderate staining. (D and H) Strong staining. All panels, original magnification, x40.

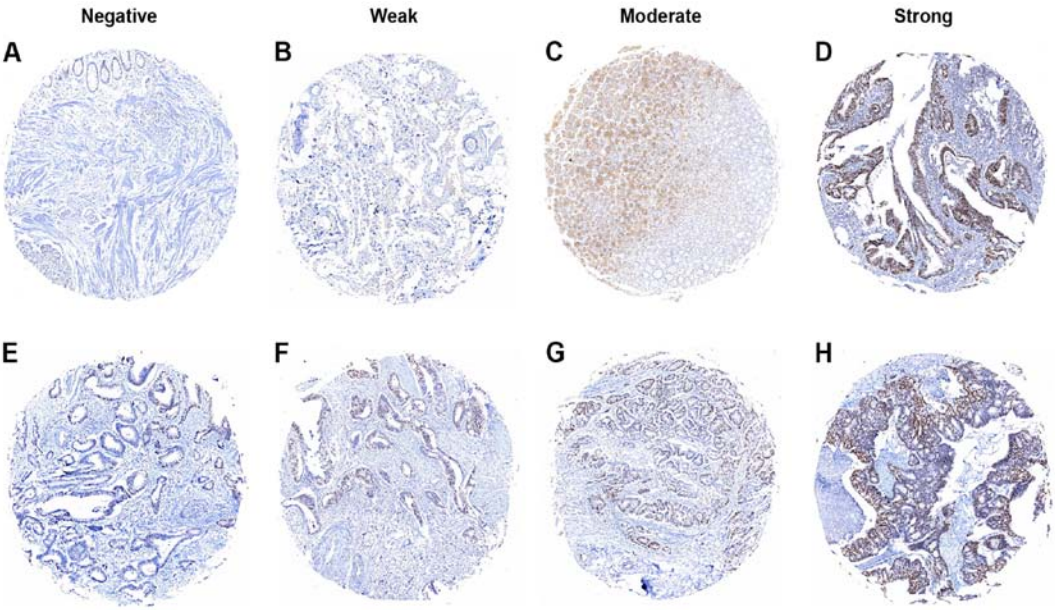

Figure 2. Representative images of c-Myc immunohistochemical staining in CRC cancer and adjacent normal tissues. (A-D) Adjacent normal tissue. (E-H) Cancer tissue. (A and E) Negative staining. (B and F) Weak staining. (C and G) Moderate staining. (D and H) Strong staining. All panels, original magnification, x40.

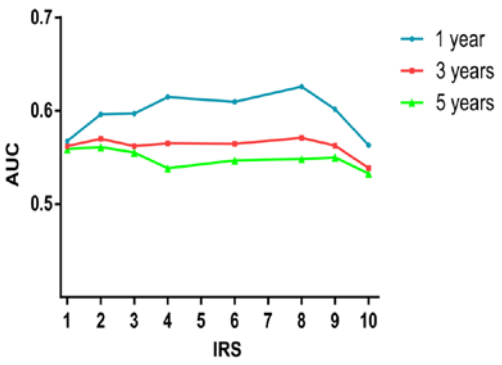

Figure 3. Area under the curve (AUC) at different cut-off values for Cul1 immunoreactivity score (IRS) for 1, 3 and 5 years of overall survival time.

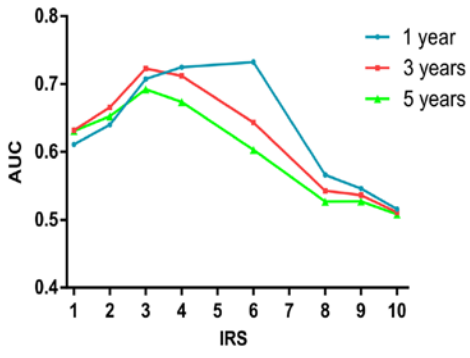

Figure 4. Area under the curve (AUC) at different cut-off values for c-Myc immunoreactivity score (IRS) for 1, 3 and 5 years of overall survival time.

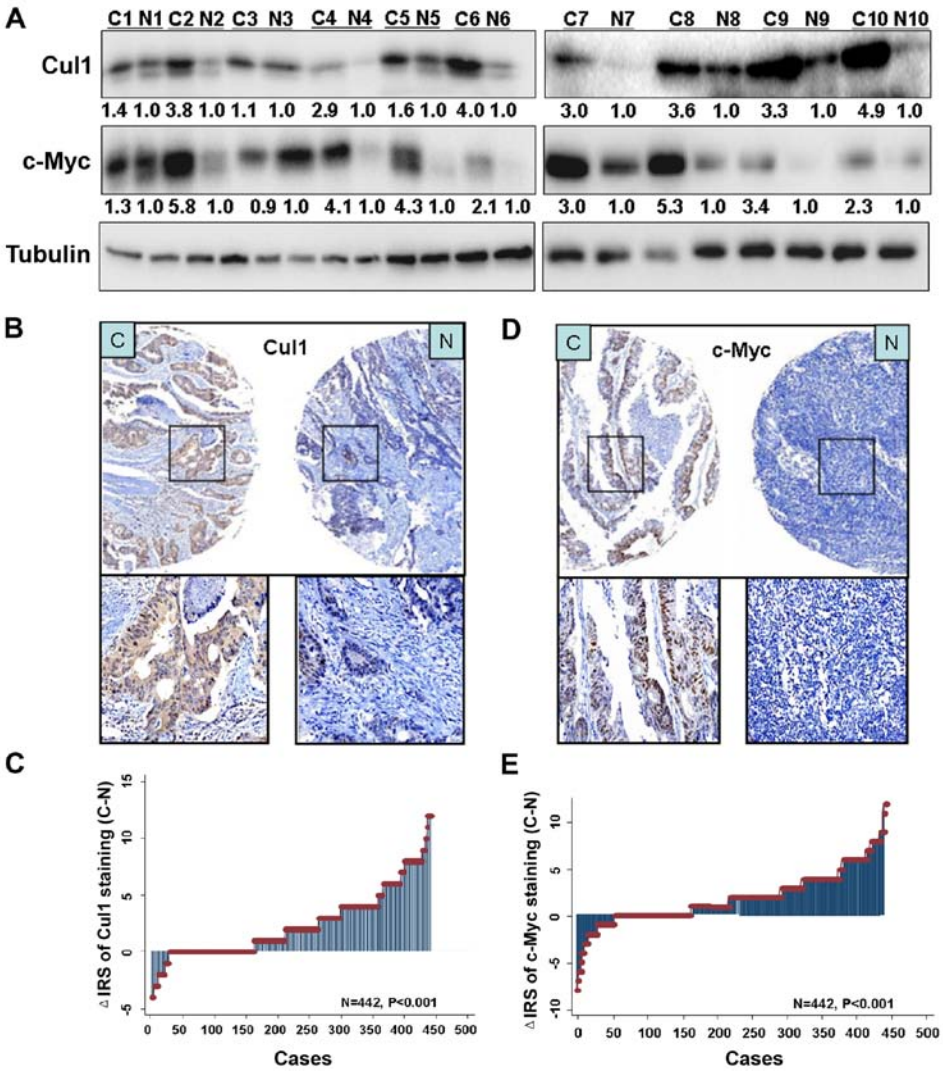

Figure 5. (A) Cul1 or c-Myc expression in CRC and adjacent normal tissues. (A) Western blotting. Representative images of expression of (B) Cul1 or (D) c-Myc in CRC and adjacent normal tissues, and IRS (C-N) for (C) Cul1 and (E) c-Myc. Top panel, original magnification, x40; bottom panel, magnification, x200. C, cancer tissue; N, adjacent normal tissue.

Statistical analysis. For TMA, statistical processing was performed using SPSS 20.0 software (SPSS, Inc, Chicago, IL, USA). Fisher's exact test was used to evaluate the association between Cul1 and c-Myc expression and clinicopathological parameters. Differences in IRS for Cul1 or c-Myc staining in primary tumors, and their paired adjacent normal tissues were assessed by the paired Wilcoxon test (raw scores). The correlation between the expression of Cul1 and c-Myc was established by Spearman rank-order correlation (raw scores) and Fisher's exact test (grouped). Probability of differences in OS as a function of time was ascertained by use of the Kaplan-Meier method, with a log-rank test probe for significance. Univariate and multivariate Cox proportional hazards regression analyses were performed to estimate the crude hazard ratios (HRs), adjusted HRs and 95% confidence interval (CI) of HRs. We evaluated the performances of different scores by plotting [t, AUC (t)] for different values of follow-up time (t). All the statistical analyses were performed by STATA statistical software (version 10.1; StataCorp, College Station, TX, USA). A P-value of <0.05 was considered statistically significant, and all tests were two-sided.

Results

Expression of Cul1 and c-Myc is increased in CRC vs. adjacent normal tissues. Ten pairs of human CRC samples, including primary CRC and matched normal colorectal tissues were collected to test the expression of Cul1 and c-Myc protein by western blotting, respectively. Data showed increased expression levels of Cul1 and c-Myc in all tumor tissues compared with the levels in the matched normal tissues (Fig. 5A). Immunohistochemical staining further confirmed higher Cul1 and c-Myc expression levels in the CRC tissues than levels in the paired adjacent normal tissues (Fig. 5B and D). There were 442 cases of CRC tissues, and with paired adjacent non-cancer tissues available for evaluating the score. As a result, Cul1 and c-Myc expression was upregulated in tumor tissues compared with that noted in the paired adjacent non-tumor tissues (P<0.001; Fig. 5C and E).

Cul1 and c-Myc expression correlates with clinicopathological parameters. In the CRC cohort, Fisher's exact analysis revealed that there was s significant positive association between high

Table II. Relationship between the expression level of Cul1 and clinicopathological features of the CRC patients.

Variables	Low n (%)	High n (%)	P-value[a]
All patients (N=464)	266 (57.3)	198 (42.7)	
Age (years)			**0.139**
≤65	157 (59.7)	106 (40.3)	
>65	109 (54.2)	92 (45.8)	
Sex			**0.175**
Male	154 (55.4)	124 (44.6)	
Female	112 (60.2)	74 (39.8)	
Pathological classification[b]			**0.302**
I	4 (80.0)	1 (20.0)	
II	242 (57.9)	176 (42.1)	
III	17 (47.2)	19 (52.8)	
Depth of invasion[b]			**0.005**
T1/T2	71 (68.9)	32 (31.1)	
T3/T4	193 (54.1)	164 (45.9)	
Lymph node metastasis[b]			**0.001**
N0	173 (63.4)	100 (36.6)	
N1/N2	91 (48.4)	97 (51.6)	
TNM stage[b]			**0.015**
I	60 (68.2)	28 (31.8)	
II	107 (60.8)	69 (39.2)	
III	88 (49.4)	90 (50.6)	
IV	8 (47.1)	9 (52.9)	
Tumor diameter (cm)[b]			**0.543**
≤5	214 (57.2)	160 (42.8)	
>5	51 (57.3)	38 (42.7)	
Distant metastasis			**0.423**
M0	256 (57.5)	189 (42.5)	
M1	10 (52.6)	9 (47.4)	
c-Myc expression			
Low	200 (72.2)	77 (27.8)	**0.001**
High	65 (34.9)	121 (65.1)	

[a]Two-sided Fisher's exact tests. [b]For some patients, these clinical pathological parameters were not available. CRC, colorectal cancer; TNM, tumor-node-metastasis. Bold indicates statistical significance.

Table III. Relationship between the expression level of c-Myc and clinicopathological features of the CRC patients.

Variables	Low n (%)	High n (%)	P-value[a]
All patients (N=464)	278 (59.9)	186 (40.1)	
Age (years)			
≤65	172 (65.4)	91 (34.6)	**0.004**
>65	106 (52.7)	95 (47.3)	
Sex			
Male	167 (60.3)	110 (39.7)	**0.458**
Female	111 (59.4)	76 (40.6)	
Pathological classification[b]			**0.091**
I	5 (100.0)	0 (0.0)	
II	251 (60.1)	167 (39.9)	
III	18 (50.0)	18 (50.0)	
Depth of invasion[b]			**<0.001**
T1/T2	77 (74.8)	26 (25.2)	
T3/T4	198 (55.5)	159 (44.5)	
Lymph node metastasis[b]			**<0.001**
N0	192 (70.3)	81 (29.7)	
N1/N2	84 (44.7)	104 (55.3)	
TNM stage[b]			**<0.001**
I	66 (75.0)	22 (25.0)	
II	121 (68.7)	55 (31.3)	
III	84 (47.2)	94 (52.8)	
IV	3 (17.7)	14 (82.3)	
Tumor diameter (cm)[b]			**0.191**
≤5	219 (58.7)	154 (41.3)	
>5	58 (64.4)	32 (35.6)	
Distant metastasis			**0.001**
M0	274 (61.6)	171 (38.4)	
M1	4 (21.1)	15 (78.9).	

[a]Two-sided Fisher's exact tests. [b]For some patients, these clinical pathological parameters were not available. CRC, colorectal cancer; TNM, tumor-node-metastasis. Bold indicates statistical significance.

Cul1 expression in cancer tissues and depth of invasion (P=0.005), lymph node metastasis (P=0.001) and TNM stage (P=0.015). However, there was no association between Cul1 expression and age, sex, pathological classification and tumor diameter (Table II).

We also analyzed the relationship between c-Myc expression and clinicopathological parameters. Data showed that high c-Myc expression in cancer tissues was significantly associated with age (P=0.004), depth of invasion (P<0.001), lymph node metastasis (P<0.001) and TNM stage (P<0.001). There was no association between c-Myc expression and sex, pathological classification and tumor diameter (Table III).

Increased Cul1 or c-Myc expression correlates with the poor survival of CRC patients. Kaplan-Meier survival assay was conducted and the data revealed that higher Cul1 and c-Myc expression in cancer tissues was correlated with a worse OS in CRC patients (P<0.001 and P<0.001, respectively, log-rank test; Fig. 6A and B). Cul1 and c-Myc expression in cancer tissues was an independent marker for the prognosis of CRC patients by univariate and multivariate Cox regression analysis. The univariate Cox regression analysis also showed that age, pathological classification, depth of invasion, lymph node metastasis, TNM stage and Cul1 or c-Myc expression were

Table IV. Univariate Cox regression analysis of Cul1 or c-Myc expression and clinicopathological variables predicting survival in CRC patients.

	n=470 cases	
Variables	HR (95% CI)	P-value
Age (≤65 vs. >65 years)	1.607 (1.215-2.126)	0.001
Sex (male vs. female)	1.013 (0.762-1.347)	0.927
Pathological classification (I/II vs. III)	2.475 (1.587-3.860)	<0.001
Depth of invasion (T1/T2 vs. T3/T4)	3.687 (2.270-5.990)	<0.001
Lymph node metastasis (N0 vs. N1/N2)	2.807 (2.112-3.731)	<0.001
TNM stage (I/II vs. III/IV)	3.214 (2.407-4.291)	<0.001
Distant metastasis (M0 vs. M1)	8.150 (4.849-13.699)	<0.001
Tumor diameter (≤5 vs. >5 cm)	1.196 (0.848-1.688)	0.307
Cul1 expression (low vs. high)	0.683 (0.515-0.905)	**0.008**
c-Myc expression (low vs. high)	0.280 (0.209-0.374)	**<0.001**
Cul1/c-Myc expression		
Both low vs. one low	1.762 (1.472-2.109)	**<0.001**
Both low vs. both high	3.422 (2.348-4.987)	**<0.001**

CRC, colorectal cancer; HR, hazard ratio; CI, confidence interval; TNM, tumor-node-metastasis. Bold indicates statistical significance.

Table V. Multivariate Cox regression analysis of Cul1, c-Myc, Cul1/c-Myc expression and clinicopathological variables predicting survival in patients with CRC.

Variables	HR (95% CI)	P-value[a]
Cul1		
Age (≤65 vs. >65 years)	1.834 (1.376-2.443)	<0.001
Sex (male vs. female)	0.925 (0.691-1.237)	0.597
Pathological classification (I/II vs. III)	1.993 (1.252-3.174)	0.004
TNM stage (I/II vs. III/IV)	3.443 (2.554-4.642)	<0.001
Tumor diameter (≤5 vs. >5 cm)	1.157 (0.805-1.662)	0.432
Cul1 expression (low vs. high)	0.757 (0.569-1.007)	**0.046**
c-Myc		
Age (≤65 vs. >65 years)	1.723 (1.289-2.302)	<0.001
Sex (male vs. female)	0.913 (0.682-1.223)	0.542
Pathological classification (I/II vs. III)	1.959 (1.227-3.129)	0.005
TNM stage (I/II vs. III/IV)	2.868 (2.117-3.887)	<0.001
Tumor diameter (≤5 vs. >5 cm)	1.309 (0.908-1.888)	0.149
c-Myc expression (low vs. high)	0.337 (0.249-0.455)	**<0.001**
Cul1/ c-Myc		
Age (≤65 vs. >65 years)	1.903 (1.432-2.529)	<0.001
Sex (male vs. female)	0.896 (0.672-1.196)	0.458
Pathological classification (I/II vs. III)	1.961 (1.234-3.167)	0.004
TNM stage (I/II vs. III/IV)	3.386 (2.522-4.546)	<0.001
Tumor diameter (≤5 vs. >5 cm)	1.159 (0.810-1.658)	0.420
Cul1/ c-Myc expression		
Both low vs. one low	2.704 (1.862-3.927)	**<0.001**
Both low vs. both high	0.073 (0.247-0.540)	**<0.001**

[a]Multivariate Cox regression analysis including age, sex, pathological classification, distant metastasis TNM stage, tumor diameter, Cul1 or c-Myc or combined expression status of the two proteins. CRC, colorectal cancer; HR, hazard ratio; CI, confidence interval; TNM, tumor-node-metastasis. Bold indicates statistical significance.

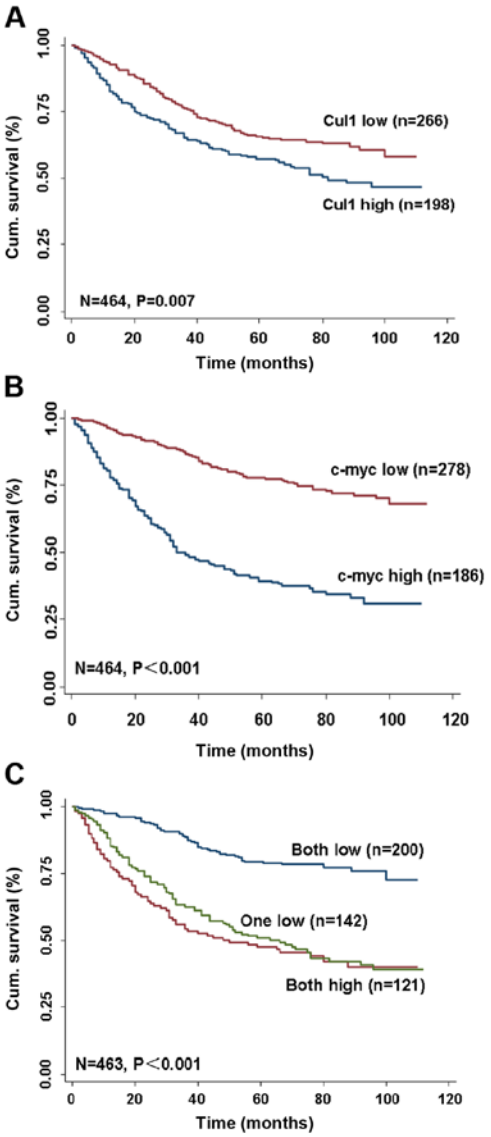

Figure 6. (A-C) Kaplan-Meier curves for overall survival (OS). (A) Cul1. (B) c-Myc. (C) Cul1 + c-Myc. P-values were calculated with the log-rank test.

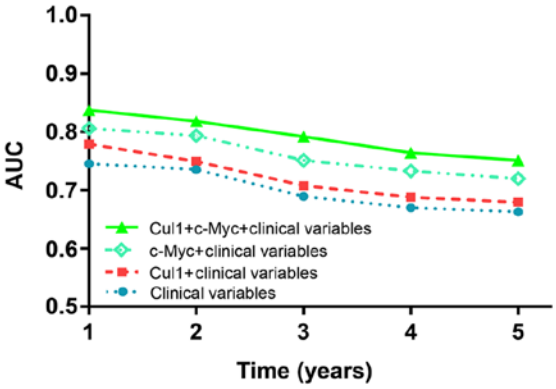

Figure 7. Time-dependent ROC analyses for clinical risk score (TNM stage, histologic type and tumor diameter), or in combination with Cul1, c-Myc or Cul1 plus c-Myc, respectively. AUC, area under the curve.

associated with OS of the CRC patients (Table IV). The multivariate Cox regression analysis revealed that Cul1 expression was an independent and unfavorable prognostic factor for CRC patients (HR, 0.749, 95% CI, 0.563-0.996, P<0.05; Table V). Similarly, c-Myc expression was also an independent and unfavorable prognostic factor for CRC patients (HR, 0.384, 95% CI 0.257-0.472, P<0.001; Table V).

Synergisic effect of Cul1 and c-Myc expression on OS in CRC patients. To further evaluate whether Cul1 combined with c-Myc has a synergetic effect on the prognosis of CRC patients, we conducted a time-dependent ROC analysis for the censored data. The data indicated that the combination of the clinical risk score (TNM stage, histologic type and tumor diameter) and Cul1 or c-Myc or Cul1 plus c-Myc expression contributed much more than any one of these markers alone in CRC patients (Fig. 7). For instance, in the TMA cohort, the AUC at year 5 was 0.663 (95% CI, 0.476-0.703) for only clinical risk score, whereas it was increased to 0.751 (95% CI, 0.499-0.748) when combined with the clinical risk score and with Cul1 plus c-Myc risk score.

The stratified analysis indicated that patients with both low expression of Cul1 and c-Myc had a more favorable outcome of survival (P<0.001; log-rank test; Fig. 6C) when compared with one low or both high expression groups. The multivariate Cox regression analysis indicated that low Cul1 and c-Myc expression alone was a favorable independent prognostic factor for CRC patients (P<0.05 for all; Table V).

Discussion

Prognostic studies of tumor biomarkers are valuable as they facilitate early diagnosis, treatment efficacy and prevention of malignancies (20). During the occurrence and development of CRC, abnormal expression of oncogenes and tumor-suppressor genes play an important role. These genes may be potentially valuable as biomarkers to determine the prognosis of CRC.

Cullin1 (Cul1) is the most representative member of the Cullin protein family, and its complex has been identified to exert ubiquitin ligase activity involved in the degradation of proteins associated with the cell cycle and cancer-associated processes. We and other investigators previously provided evidence that Cul1 overexpression is associated with the poor prognosis of gastric (10), breast (13) and non-small cell lung cancer (21). In the present study, we provided new evidence that Cul1 expression is higher in CRC tumor tissues compared with that observed in matched adjacent normal tissues. We also demonstrated that high Cul1 expression in CRC tumor tissues was significantly correlated with depth of invasion, lymph node metastasis and TNM stage. In addition, Kaplan-Meier survival analysis revealed that high Cul1 expression in tumor tissues was correlated with poor OS in CRC patients. Furthermore, univariate and multivariate Cox proportional hazards regression analyses showed that Cul1 expression is an independent negative prognostic factor of CRC.

c-Myc is a well known oncogene and plays a vital role in the developmental process of many types of cancers (22). c-Myc was found to promote cell proliferation, accelerate the cell cycle, inhibit cell differentiation and induce apoptosis by activating the PTEN/PI3K/AKT pathway (23). c-Myc is highly expressed in CRC and promotes the malignant proliferation

of tumor cells (17); c-Myc gene copy-number was identified as an independent factor for poor prognosis in CRC (24,25). c-Myc overexpression is associated with a worse prognosis in prostate cancer (26), lymphoma (27,28), lung (29) and breast cancer (30). Nevertheless, few studies have examined the clinicopathological and prognostic implications of c-Myc status in CRC. In the present study, c-Myc expression was upregulated in tumor tissues compared with that noted in the paired adjacent non-tumor tissues in both CRC fresh tissues and a TMA cohort. We demonstrated that high c-Myc expression in CRC cancer tissures was significantly correlated with age, depth of invasion, lymph node metastasis and TNM stage. High c-Myc expression was also correlated with worse OS and was found to be an independent negative prognostic factor in CRC patients. Our cohort indicated that increased expression of Cul1 and c-Myc was significantly associated with unfavorable clinicopathologic parameters and worse OS for CRC. c-Myc enhances expression of Cul1 and promotes ubiquitin-dependent proteolysis and cell cycle progression (18). On the contrary, Cul1 assembled SCF (Skp1/Cul1/F-box)^{FBXO28} plays an important role in the regulation of MYC-driven cancers (31). In the present study, we also demonstrated that Cul1 combined with c-Myc has synergistic potential and may be more effective than Cul1 or cMyc alone in predicting the prognosis of CRC patients.

In conclusion, our findings indicate that Cul1 and c-Myc are unfavorable prognostic factors for CRC patients. In addition, to the best of our knowledge, we first revealed the combined value of Cul1 and c-Myc as efficient prognostic factors. Although the co-action of these two proteins can be used to predict the prognosis of CRC, further investigation is warranted to elucidate their role in the occurrence and development of CRC.

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