CIP4 Expression is Associated With The Prognosis in Colorectal Cancer

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Abstract

Background: This study was conducted to detect the expression of Cdc42 interacting protein 4 (*CIP4*) in patients with colorectal cancer (CRC), and explore the role of *CIP4* in prognosis of CRC patients.

Methods: The expression of *CIP4* mRNA was determined by quantitative real-time PCR (qRT-CPR) and compared by student's t-test between groups. Relationships of clinical characteristics and *CIP4* expression were analyzed by Chi-square test. Kaplan-Meier curves were used to estimate the overall survival of CRC patients. And Cox regression analysis was conducted to identify the prognostic biomarkers for CRC patients.

Results: The qRT-PCR results showed that CRC tissues were detected with significantly high *CIP4* mRNA expression compared with adjacent normal controls ($P<0.0001$). The overexpression of *CIP4* in CRC tissues was influenced by distant metastasis ($P=0.021$), lymphatic invasion ($P=0.012$) and TNM stage ($P=0.006$). But, other clinical factors including age, gender, differentiation and tumor site were proved to have no obvious effects on *CIP4* expression (all, $P>0.05$). The survival curves showed that patients with high *CIP4* expression generally lived shorter than those with low *CIP4* expression ($P<0.001$). In addition, the multivariate analysis revealed that differentiation ($P=0.044$, HR=1.631, 95%CI=1.013-2.626) and *CIP4* expression ($P=0.000$, HR=5.283, 95%CI=3.138-8.893) were of great prognostic significance for CRC patients.

Conclusion: Taken together, up-regulation of *CIP4* in CRC tissues represented poor prognosis for patients.

Background

Colorectal cancer (CRC) is one of the most common malignancies worldwide with increasing incidence rate, especially in developed counties [1, 2]. According to the global cancer statistics, CRC is proved to be the third common type of cancer and the fourth leading cause of cancer-related death in the world [3, 4]. Moreover, it has been reported that there are more than 1 million newly diagnosed CRC cases annually and CRC is responsible for more than 600,000 death cases each year in the word [5, 6]. At present, the treatment strategies for CRC patients are mainly surgical resection, which is the optimal method [7, 8]. However, the prognosis of CRC patients is still significantly poor because of the recurrence, metastasis and advanced stages [9, 10]. The 5-year overall survival rate of CRC patients has been claimed to be less than 60%, and even 10–15% in certain metastasis cases [11, 12]. Although some prognostic factors have been used for the prognosis of CRC, the survival time varies widely in patients with different TNM stage and grade. As a result, it is essential to find efficient biomarker to predict and treat CRC.

Cdc42-interacting protein 4 (*CIP4*), also known as *TRIP10*, is a member of the F-BAR (Fes-CIP4 homology-Bin/Amphyphysin/Rvs) protein family, which consists of a N-terminal F-BAR domain, a HR1 (PKN homology region-1) domain and a C-terminal SH3 (SRC homology 3) domain [13, 14]. It has been demonstrated that CIP4 protein is composed of 545 amino acids, interacts with Cdc42 protein as a downstream effector of Cdc42 [15]. What's more, numerous studies have suggested that *CIP4* is

implicated in a variety of biological regulation progresses, such as allergic response, glucose metabolism, membrane deformation and tubulation, endocytosis, vesicle scission and remodeling of actin cytoskeleton [16–18]. The aberrant expression of *CIP4* has been investigated in various cancer, including breast cancer and CRC [16, 19]. However, the clinical role of *CIP4* in CRC prognosis was still unclear.

In the present study, we were engaged in determining the expression of *CIP4* in CRC and estimating its prognosis value in patients with this disease.

Methods

Patients and specimens

A total of 117 CRC patients who were subjected to surgical resection in the Southwest Hospital, Army Medical University were enrolled in our study. Patients with preoperative chemotherapy or radiotherapy were excluded from our investigation. Clinical information of the patients was recorded, including age, gender, differentiation, tumor size, distant metastasis, TNM stage and lymphatic invasion. The CRC tissue samples and the adjacent non-carcinoma tissues were obtained by surgery and immediately put into liquid nitrogen then stored at -80℃ for use. All patients were followed up through telephone calls in a 5-year duration. Moreover, the study was supported by the Ethics Committee of Southwest Hospital, Army Medical University. And each patient had provided the written the informed consents in advance.

Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from CRC tissues and adjacent normal controls with Trizol reagent (Invitrogen) following the manufacture's instructions. Then the first strand of cDNA was synthesized by iScript cDNA Synthesis Kit (BioRad). Finally quantitative real-time PCR was conducted using iQ SYBR Green reagent (BioRad) with a MyiQ Color Real-Time PCR Detection System (BioRad). Expression of *CIP4* mRNA was normalized to *GAPDH*. Each sample was treated in triplicate.

Statistical analysis

All analyses were carried out with SPSS 18.0 (SPSS, Inc., Chicago, IL, USA) and Sigmaplot 12.5 (Systat Software Inc.) softwares. The difference of *CIP4* expression in CRC tissues and controls was compared using the student's t-test. Relationships of clinical factors with *CIP4* expression were analyzed by Chi-square test. The overall survival rate of patients was analyzed by Kaplan-Meier survival curves. And the Cox regression analysis was used to evaluate the prognostic significance of clinical factors in CRC. The results were considered to be statistically significant when *P* was less than 0.05.

Results

Increased expression of *CIP4* mRNA in CRC tissues

The expression of *CIP4* mRNA was determined in 117 pairs of CRC tissue samples and controls using qRT-PCR. As shown in Fig. 1, the expression level of *CIP4* mRNA was significantly higher in CRC tissues than in the controls (3.42 ± 0.80 vs 1.91 ± 0.51). Significant difference was found between the two groups ($P < 0.0001$).

Association between *CIP4* expression and clinical characteristics of CRC patients

According to the median expression level of *CIP4* (3.45), all patients were divided into two groups manually: the high expression group (n = 59) and the low expression group (n = 58). As shown in Table 1, overexpression of *CIP4* was significantly related with distant metastasis ($P = 0.021$), lymphatic invasion ($P = 0.012$) and TNM stage ($P = 0.006$). However, no significant relationship was observed between *CIP4* expression and other clinical parameters, including age ($P = 0.074$), gender ($P = 0.079$), differentiation ($P = 0.079$) and tumor size ($P = 0.116$).

Table 1
Relationship of *CIP4* expression and clinical characteristics of CRC patients

Clinical features	Case NO.(n = 117)	*CIP4* Expression		χ^2	*P* value
		High	Low		
Age (years)				3.201	0.074
≦ 50	67	29	38		
> 50	50	30	20		
Gender				3.077	0.079
Female	55	23	32		
Male	62	36	26		
Differentiation				3.089	0.079
Poor	58	34	24		
Moderate, well	59	25	34		
Tumor site				2.469	0.116
Colon	52	22	30		
Rectum	65	37	28		
Distant metastasis				5.341	0.021
Negative	54	21	33		
Positive	63	38	25		
Lymphatic invasion				6.262	0.012
Absent	61	24	37		
Present	56	35	21		
TNM stage				7.466	0.006
I,II	68	27	41		
III,IV	49	32	17		

Correlation between *CIP4* expression and survival of CRC patients

The correlation between *CIP4* expression and survival of CRC patients was analyzed by Kaplan-Meier curve and Cox regression analysis. During the 5-year follow-up, 22 out of 58 (37.93%) patients with low *CIP4* expression died, and 49 out of 59 (83.05%) patients with high *CIP4* expression died. As shown in Fig. 2, patients with high expression of *CIP4* had lower survival time than those with low *CIP4* expression

($P < 0.001$). In addition, Cox univariate analysis suggested that differentiation, distant metastasis, lymphatic invasion, TNM stage and *CIP4* expression were related with prognosis of CRC patients (Table 2). Furthermore, the multivariate analysis further revealed that differentiation ($P = 0.044$, HR = 1.631, 95%CI = 1.013–2.626) and *CIP4* expression ($P = 0.000$, HR = 5.283, 95%CI = 3.138–8.893) were two independent biomarkers for CRC patients prognosis.

Table 2
Univariate and multivariate analyses of clinical features in CRC patients

Clinical features	Univariate		Multivariate	
	P value	HR (95%CI)	*P* value	HR (95%CI)
Differentiation	0.011	1.849 (1.151–2.971)	0.044	1.631 (1.013–2.626)
Distant metastasis	0.017	1.792 (1.108–2.897)	-	-
Lymphatic invasion	0.027	1.696 (1.061–2.713)	-	-
TNM stage	0.007	1.905 (1.194–3.039)	-	-
CIP4 expression	0.000	5.441 (3.253-9.100)	0.000	5.046 (3.001–8.485)

Discussion

CRC is one of the most common malignant tumors in the world with increasing incidence rate year by year [20, 21]. In recent years, with the progress of surgical treatment and the advent of new chemotherapy drugs as well as the popularity of endoscopic diagnosis technology, the curative effects of patients are significantly improved to a certain extent. However the 5-year survival of patients is still unsatisfied, especially for those with advanced stage. This is mainly caused by lack of knowledge about survival-related factors and lack reasonable prognostic evaluation system. It is apparent that the understanding on tumors has been deepened with the development of molecular biology. Therefore, the molecular biology will be benefit for early diagnosis and prevention of CRC to explore the prognostic biomarkers and treat these markers with positive intervention.

CIP4 is a skeleton protein of CDC42, which is widely present in human organs, such as brain, trachea, liver, kidney, colon, heart, lung and prostate. It has been revealed that *CIP4* is involved in the process of epithelial-mesenchymal transition (EMT), which is important for the embryonic development, chronic inflammation and cancer metastasis, through regulating the endocytosis of E-cadherin via different signaling pathways [22, 23]. Besides, *CIP4* is reported to mainly regulate the polymerization of actin and dynamics of cell membrane and to stabilize the tonofilaments to recombine ctyoskeleton. Moreover, *CIP4* also plays an important role in cell morphology, cell polarity, cell adhesion, intracellular transport, and signal transduction. So far, up-regulation of *CIP4* has been investigated in various diseases, indicating *CIP4* might be related with disease progression. For example, Malet-Engra et al. showed that the expression of *CIP4* was at high level in chronic lymphocytic leukemia [24]. In the study of Otto et al., they

found that in human invasive breast cancer, high *CIP4* level was significantly associated with tumor progression and promoting disease metastasis [25].

In the present study, we determined the expression of *CIP4* in CRC tissues and then investigated its role in prognosis of CRC patients. The expression of *CIP4* in CRC samples was significantly higher than that in paired normal controls. Besides, the Chi-square test demonstrated that *CIP4* overexpression was closely related with distant metastasis, lymphatic invasion and advanced TNM stage. The above results confirmed the previous assumption, indicating *CIP4* might be involved in the development and progression of CRC. Based on the above results and hypothesis, we further explored the prognostic significance of *CIP4* for CRC patients. The survival curves showed that patients with high *CIP4* level were more easily to die than those with low *CIP4* expression, concluding that *CIP4* up-regulation represented unfavorable prognoses in CRC. Meanwhile, the Cox regression analysis suggested *CIP4* expression was a prognostic marker for CRC patients.

It is well known that invasion and metastasis are two important features of malignant tumors, and also main causes for cancer-related deaths. There are evidences proving that *CIP4* expression is significantly related with cell metastasis and invasion. Truesdull et al. demonstrated that *CIP4* expression was greatly elevated and could promote tumor metastasis in lung adenocarcinoma [26]. Besides previous study also proved that *CIP4* expression was significantly elevated and could promote cell metastasis in triple-negative breast cancer [25]. These might provide theoretical foundations for us to further investigate the mechanisms of *CIP4* on CRC development and progression.

Conclusions

In conclusion, *CIP4* was highly expressed in CRC tissues compared with paired normal controls. Up-regulation of *CIP4* was significantly related to distant metastasis, lymphatic invasion and TNM stage. From the survival curve, patients with low *CIP4* expression had more favorable survival than those with high *CIP4* expression. Cox regression analysis revealed *CIP4* expression was a promising candidate marker for CRC prognosis.

Abbreviations

Cdc42 interacting protein 4 (CIP4)

Colorectal cancer (CRC)

Quantitative real-time PCR (qRT-CPR)

Epithelial-mesenchymal transition (EMT)

Declarations

Ethics approval and consent to participate

This study was supported by the Ethics Committee of Southwest Hospital, Army Medical University and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

The subjects had been informed the objective. Certainly, written consents were signed by every subject in this study.

Consent for publication

We obtaining permission from participants to publish their data.

Availability of data and materials All data generated or analysed during this study are included in this published article.**Competing interests**The authors declare that they have no competing interests.**Authors' contributions** K.Z. and Z.W. design of the work; T.M. and Z.H. the acquisition, analysis, X.W. and Y.P. interpretation of data; Y.C. and Y.D. the creation of new software used in the work; Z.R. have drafted the work or substantively revised it. All authors read and approved the final manuscript.

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Figures

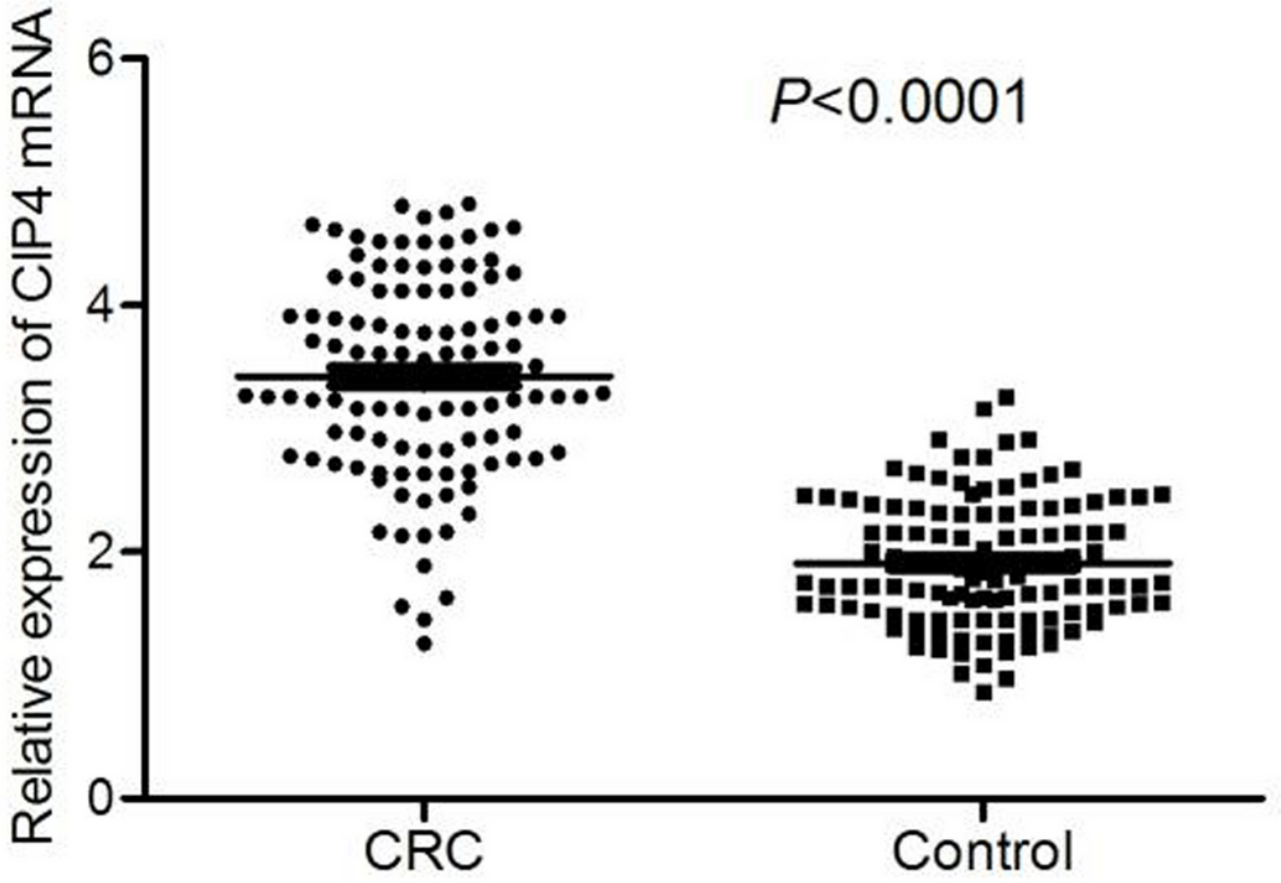
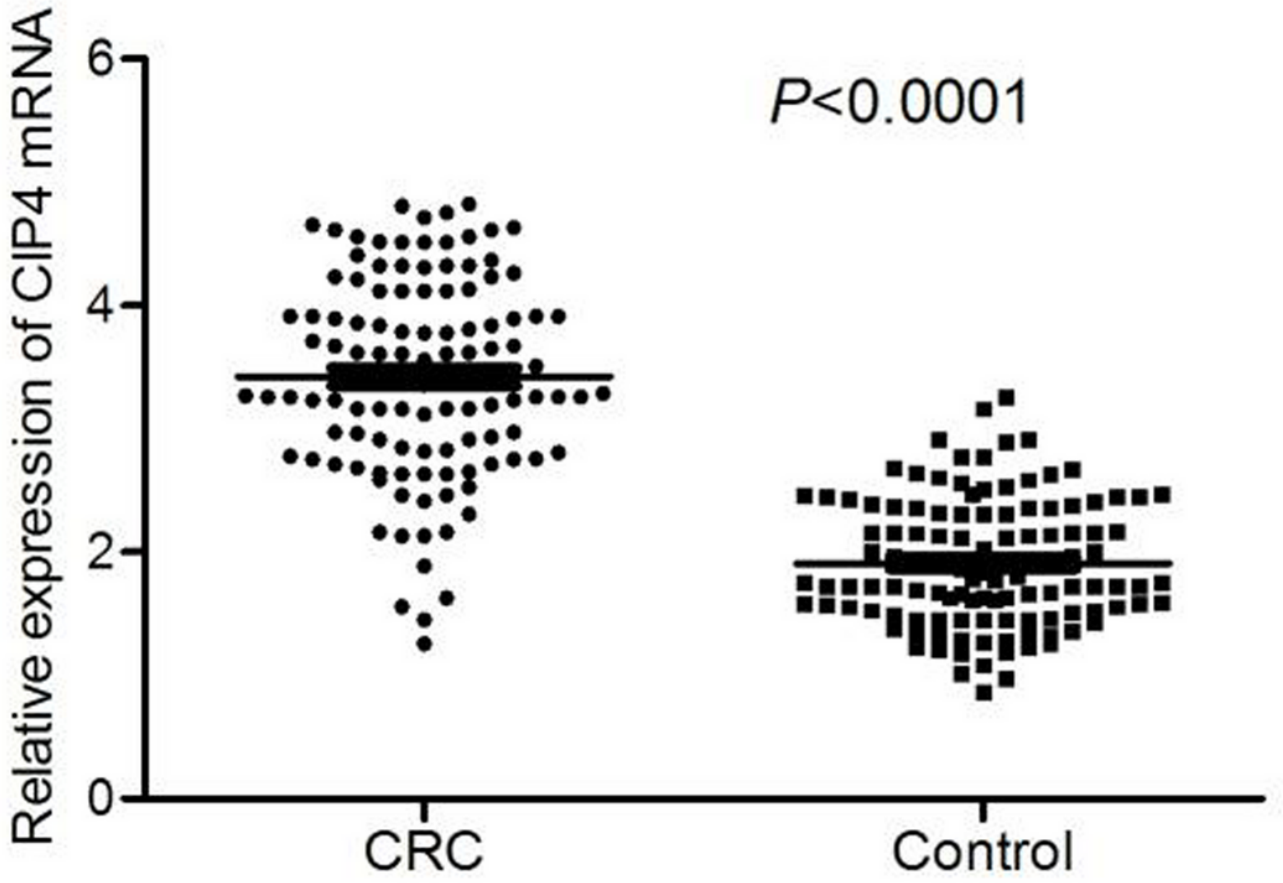


Figure 1

Expression of CIP4 mRNA was detected by qRT-PCR in CRC tissues and paired normal controls. The result claimed a higher level of CIP4 mRNA in CRC tissues than normal controls (P<0.0001).



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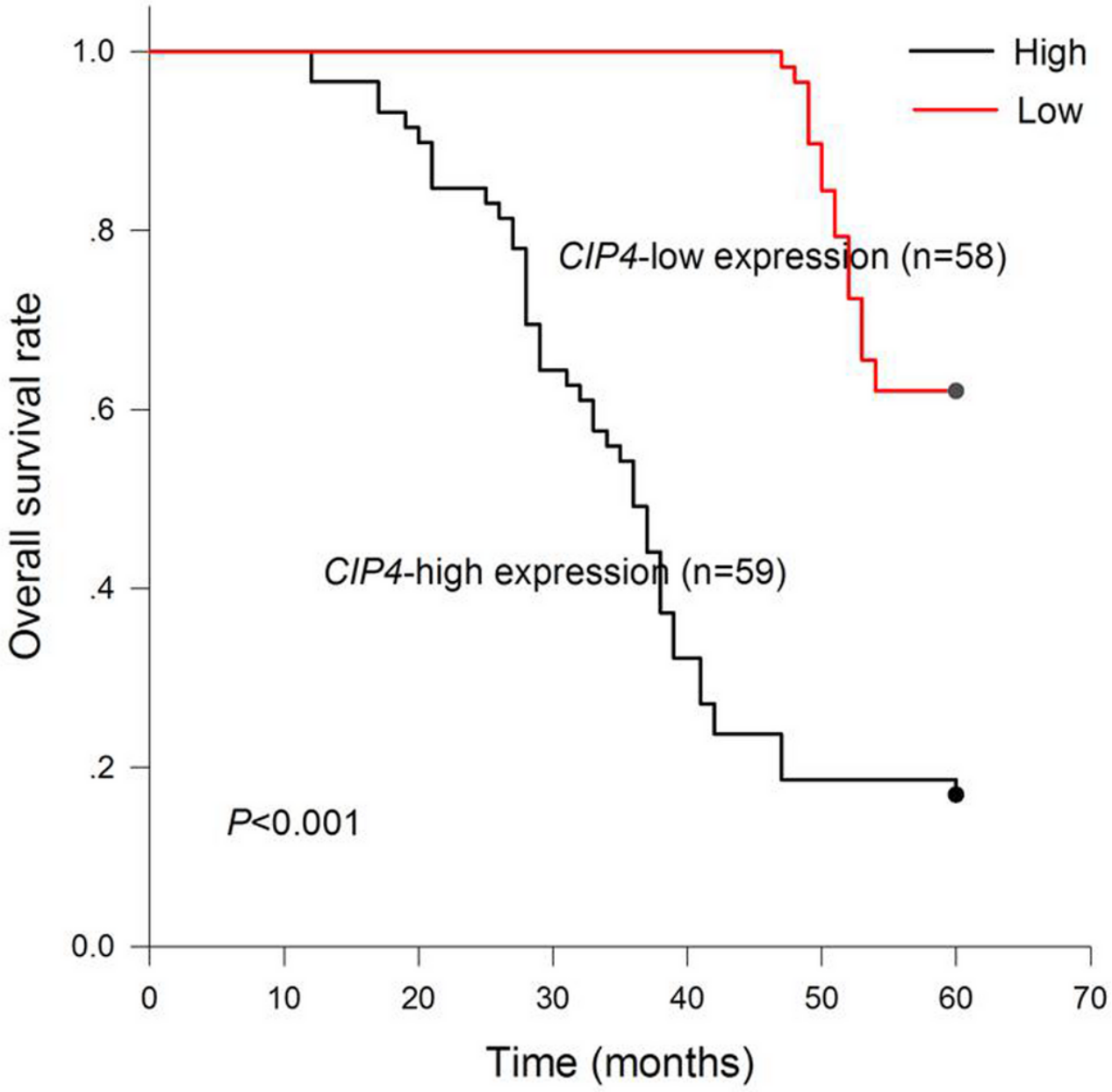
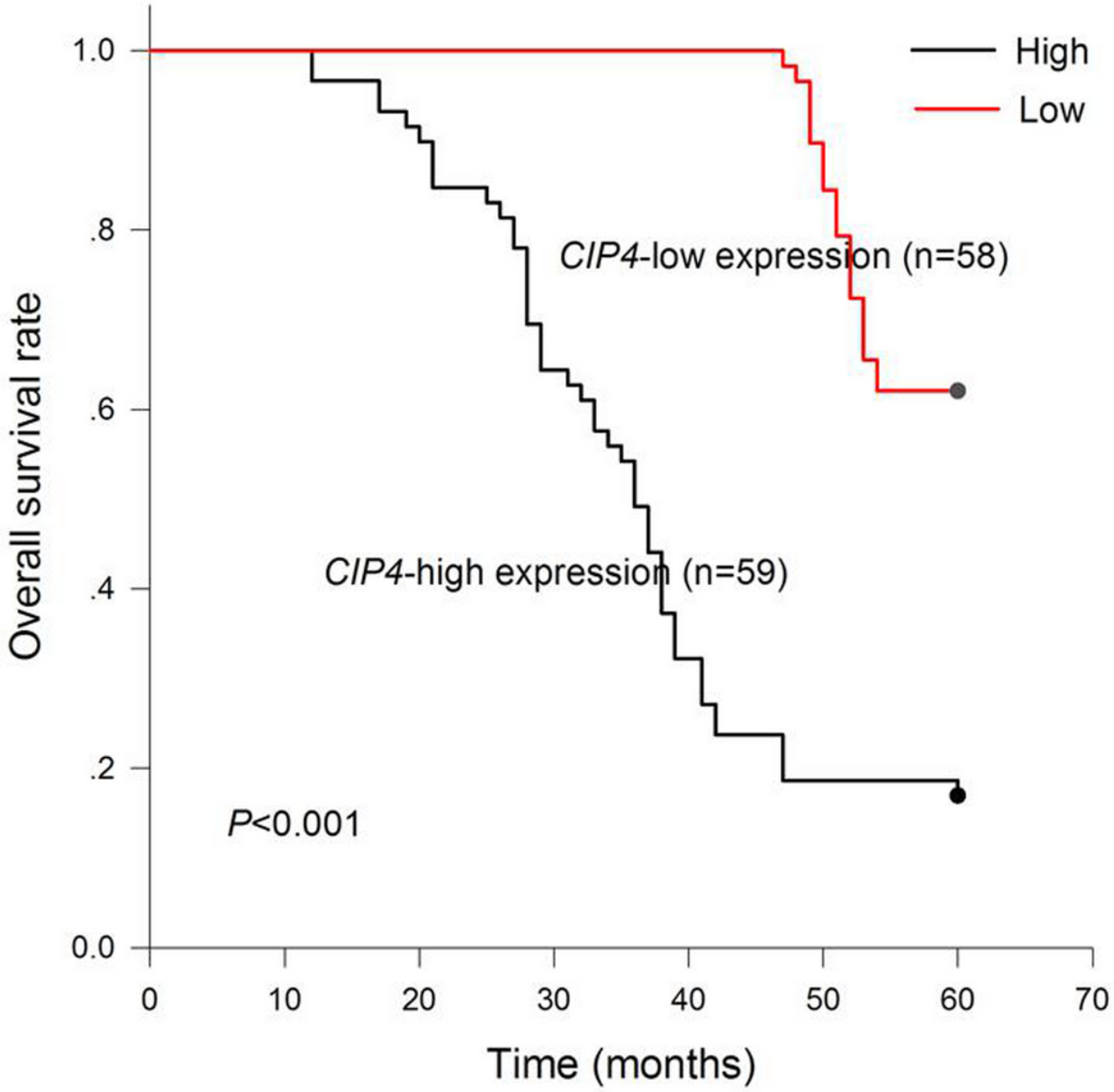


Figure 2

Kaplan-Meier survival curves were plotted to elucidate the overall survival of CRC patients with different CIP4 expression. It was observed that patients with high CIP4 expression had a significant lower overall survival rate than those with low CIP4 expression (P<0.001).



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