CIP4 Expression is Associated With The Prognosis in Colorectal Cancer

Keqian Zhang  
Southwest Hospital, Army Medical University

Tianqi Mao  
Southwest Hospital, Army Medical University

Zhicheng He  
Southwest Hospital, Army Medical University

Xiaojiao Wu  
Southwest Hospital, Army Medical University

Yu Peng  
Southwest Hospital, Army Medical University

Yanrong Chen  
Southwest Hospital, Army Medical University

Yan Dong  
Southwest Hospital, Army Medical University

Zihua Ruan  
Southwest Hospital, Army Medical University

Zhe Wang (✉ dsfgfy@126.com)  
Southwest Hospital, Army Medical University  https://orcid.org/0000-0003-3185-612X

Primary research

Keywords: CIP4, Colorectal cancer, Prognosis, Survival

DOI: https://doi.org/10.21203/rs.3.rs-95046/v1

License: ☑️  This work is licensed under a Creative Commons Attribution 4.0 International License.  
Read Full License
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 world [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 \textit{CIP4} has been investigated in various cancer, including breast cancer and CRC [16, 19]. However, the clinical role of \textit{CIP4} in CRC prognosis was still unclear.

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

\section*{Methods}

\subsection*{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\degree C 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 informed consents in advance.

\subsection*{Quantitative real-time PCR (qRT-PCR)}

Total RNA was extracted from CRC tissues and adjacent normal controls with Trizol reagent (Invitrogen) following the manufacturer'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 \textit{CIP4} mRNA was normalized to \textit{GAPDH}. Each sample was treated in triplicate.

\section*{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 \textit{CIP4} expression in CRC tissues and controls was compared using the student's t-test. Relationships of clinical factors with \textit{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.

\section*{Results}

Increased expression of \textit{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).
| Clinical features          | Case NO. (n = 117) | CIP4 Expression | \(\chi^2\) | \(P\) value |
|---------------------------|-------------------|-----------------|------------|-------------|
|                           |                   | High | Low |        |            |
| Age (years)               |                   | 3.201 |    | 0.074 |
| \(\leq 50\)              | 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.
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 \((P=0.044, \text{HR}=1.631, 95\%\text{CI}=1.013-2.626)\) and \( CIP4 \) expression \((P=0.000, \text{HR}=5.283, 95\%\text{CI}=3.138-8.893)\) were two independent biomarkers for CRC patients prognosis.

### Table 2

| 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 cytoskeleton. 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.

Acknowledgements Not applicable.

References

1. Xu B, Yu L, Zhao LZ, Ma DW. Prognostic factors in the patients with T2N0M0 colorectal cancer. World J Surg Oncol. 2016;14:76.
2. Hoekstra E, Das AM, Swets M, Cao W, van der Woude CJ, Bruno MJ, Peppelenbosch MP, Kuppen PJ, Ten Hagen TL, Fuhler GM. Increased PTP1B expression and phosphatase activity in colorectal cancer results in a more invasive phenotype and worse patient outcome. Oncotarget. 2016;7(16):21922–38.
3. Ma W, Yu Q, Jiang J, Du X, Huang L, Zhao L, Zhou QL. miR-517a is an independent prognostic marker and contributes to cell migration and invasion in human colorectal cancer. Oncology letters. 2016;11(4):2583–9.
4. Kawaguchi K, Senga S, Kubota C, Kawamura Y, Ke Y, Fujii H. High expression of Fatty Acid-Binding Protein 5 promotes cell growth and metastatic potential of colorectal cancer cells. FEBS open bio. 2016;6(3):190–9.
5. You J, Zhu GQ, Xie L, Liu WY, Shi L, Wang OC, Huang ZH, Braddock M, Guo GL, Zheng MH. Preoperative platelet to lymphocyte ratio is a valuable prognostic biomarker in patients with colorectal cancer. Oncotarget. 2016;7(18):25516–27.
6. Cai ZZ, Xu JG, Zhou YH, Zheng JH, Lin KZ, Zheng SZ, Ye MS, He Y, Liu CB, Xue ZX. Human cytomegalovirus-encoded US28 may act as a tumor promoter in colorectal cancer. World journal of gastroenterology: WJG. 2016;22(9):2789–98.
7. Torabizadeh Z, Nosrati A, Tahvildari S. Human Epidermal Growth Factor Receptor Expression in Colorectal Cancer and Its Relationship with Clinicopathological Characteristics. Middle East journal of digestive diseases. 2016;8(1):24–30.

8. Hachimaru A, Maeda R, Suda T, Takagi Y. Repeat pulmonary resection for recurrent lung metastases from colorectal cancer: an analysis of prognostic factors. Interact Cardiovasc Thorac Surg. 2016;22(6):826–30.

9. Li G, Wang Z, Xu J, Wu H, Cai S, He Y: The prognostic value of lactate dehydrogenase levels in colorectal cancer: a meta-analysis. BMC cancer 2016, 16:249.

10. Liu T, Zhang X, Yang YM, Du LT, Wang CX: Increased expression of the long noncoding RNA CRNDE-h indicates a poor prognosis in colorectal cancer, and is positively correlated with IRX5 mRNA expression. Onco Targets and therapy 2016, 9:1437–1448.

11. Fan C, Lin Y, Mao Y, Huang Z, Liu AY, Ma H, Yu D, Maitikabili A, Xiao H, Zhang C, et al. MicroRNA-543 suppresses colorectal cancer growth and metastasis by targeting KRAS, MTA1 and HMGA2. Oncotarget. 2016;7(16):21825–39.

12. Jia M, Gao X, Zhang Y, Hoffmeister M, Brenner H. Different definitions of CpG island methylator phenotype and outcomes of colorectal cancer: a systematic review. Clinical epigenetics. 2016;8:25.

13. Chen Y, Aardema J, Kale S, Whichard ZL, Awomolo A, Blanchard E, Chang B, Myers DR, Ju L, Tran R, et al. Loss of the F-BAR protein CIP4 reduces platelet production by impairing membrane-cytoskeleton remodeling. Blood. 2013;122(10):1695–706.

14. Saengsawang W, Taylor KL, Lumbard DC, Mitok K, Price A, Pietila L, Gomez TM, Dent EW. CIP4 coordinates with phospholipids and actin-associated proteins to localize to the protruding edge and produce actin ribs and veils. Journal of cell science. 2013;126(Pt 11):2411–23.

15. Xu C, Zhou Q, Liu L, Liu P, Pei G, Zeng R, Han M, Xu G. Cdc42-Interacting Protein 4 Represses E-Cadherin Expression by Promoting beta-Catenin Translocation to the Nucleus in Murine Renal Tubular Epithelial Cells. Int J Mol Sci. 2015;16(8):19170–83.

16. Pichot CS, Arvanitis C, Hartig SM, Jensen SA, Bechill J, Marzouk S, Yu J, Frost JA, Corey SJ. Cdc42-interacting protein 4 promotes breast cancer cell invasion and formation of invadopodia through activation of N-WASp. Cancer research. 2010;70(21):8347–56.

17. Tonucci FM, Hidalgo F, Ferretti A, Almada E, Favre C, Goldenring JR, Kaverina I, Kierbel A, Larocca MC. Centrosomal AKAP350 and CIP4 act in concert to define the polarized localization of the centrosome and Golgi in migratory cells. Journal of cell science. 2015;128(17):3277–89.

18. Rusconi F, Thakur H, Li J, Kapiloff MS. CIP4 is required for the hypertrophic growth of neonatal cardiac myocytes. Journal of biomedical science. 2013;20:56.

19. Hu ZY, Liu YP, Xie LY, Wang XY, Yang F, Chen SY, Li ZG. AKAP-9 promotes colorectal cancer development by regulating Cdc42 interacting protein 4. Biochim Biophys Acta. 2016;1862(6):1172–81.

20. Han D, Gao X, Wang M, Qiao Y, Xu Y, Yang J, Dong N, He J, Sun Q, Lv G, et al. Long noncoding RNA H19 indicates a poor prognosis of colorectal cancer and promotes tumor growth by recruiting and
21. Xu T, Zong Y, Peng L, Kong S, Zhou M, Zou J, Liu J, Miao R, Sun X, Li L. Overexpression of eIF4E in colorectal cancer patients is associated with liver metastasis. OncoTargets therapy. 2016;9:815–22.

22. Wirtz-Peitz F, Zallen JA. Junctional trafficking and epithelial morphogenesis. Curr Opin Genet Dev. 2009;19(4):350–6.

23. Bai S, Zeng R, Zhou Q, Liao W, Zhang Y, Xu C, Han M, Pei G, Liu L, Liu X, et al. Cdc42-interacting protein-4 promotes TGF-Beta1-induced epithelial-mesenchymal transition and extracellular matrix deposition in renal proximal tubular epithelial cells. Int J Biol Sci. 2012;8(6):859–69.

24. Malet-Engra G, Viaud J, Ysebaert L, Farce M, Lafouresse F, Laurent G, Gaits-Iacovoni F, Scita G, Dupre L. CIP4 controls CCL19-driven cell steering and chemotaxis in chronic lymphocytic leukemia. Cancer research. 2013;73(11):3412–24.

25. Cerqueira OL, Truesdell P, Baldassarre T, Vilella-Arias SA, Watt K, Meens J, Chander H, Osorio CA, Soares FA, Reis EM, et al. CIP4 promotes metastasis in triple-negative breast cancer and is associated with poor patient prognosis. Oncotarget. 2015;6(11):9397–408.

26. Truesdell P, Ahn J, Chander H, Meens J, Watt K, Yang X, Craig AW. CIP4 promotes lung adenocarcinoma metastasis and is associated with poor prognosis. Oncogene. 2015;34(27):3527–35.

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).
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).