

# Overexpression of mRNA-decapping enzyme 1a affects survival rate in colorectal carcinoma

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Received February 11, 2017; Accepted February 2, 2018

DOI: 10.3892/ol.2018.8730

**Abstract.** Processing bodies (P-bodies) are one of the most well understood types of RNA granules, and are associated with a variety of diseases, including cancer. mRNA-decapping enzyme 1a (DCP1a), which may be used as a marker to analyze P-bodies, participates in the removal of the 5'-methylguanosine cap from eukaryotic mRNAs as a cofactor. The aim of the present study was to analyze the association between DCP1a expression and clinical features in colorectal carcinoma (CRC). The levels of DCP1a mRNA expression were detected by reverse transcription-quantitative polymerase chain reaction assay in carcinoma and non-carcinoma tissues from 75 patients, while the protein expression levels were evaluated by immunohistochemistry and western blotting. Additional associations between DCP1a expression and clinical characteristics were analyzed by  $\chi^2$  test and Cox regression analysis. In the 75 cases, the levels of DCP1a mRNA and protein expression were increased in colorectal carcinoma tissues compared with non-carcinoma tissues. A high expression of DCP1a was significantly associated with lower survival rates in patients with CRC compared with patients with low DCP1a expression ( $P=0.001$ ). Associations with depth of invasion ( $P=0.008$ ), lymph node metastasis ( $P=0.001$ ) and tumor node metastasis stage ( $P=0.001$ ) were also observed. Additional Cox regression analysis revealed that the DCP1a expression ( $P=0.012$ ) is an independent factor in survival rate. It was also identified that DCP1a may have high expression in colorectal carcinoma tissues and be associated with poor prognosis. This suggests that DCP1a may be a diagnostic marker or prognostic indicator to assist with patient assessments and therapies.

## Introduction

In spite of previous efforts, colorectal cancer (CRC) remains one of the most prevalent cancer types worldwide, and North America and Europe have the highest morbidity rates worldwide (1,2). In the United States of America alone, there were >130,000 estimated cases, and >40,000 estimated mortalities due to this disease in 2016, ranking third in mortality in male and female populations (3). Concurrently, as a result of the effects of the Western lifestyle, the morbidity rate is also increasing in developing countries. In China, for example, the estimated incidence and mortality rates of CRC were ranked fifth among all malignant diseases in 2015 (4). Due to the high incidence rate and poor prognosis, the exploration of sensitive diagnostic methods and prognostic indicators is required.

mRNA regulation serves a key role in modulating gene expressions in cells (5,6). As previously established, decapping is an essential step in regulating mRNA degradation in eukaryotes (7). mRNA-decapping enzyme 1a (DCP1a) is a cofactor involved in the removal of the 5'-methylguanosine cap from eukaryotic mRNAs, and may be used as a marker to analyze cellular processing bodies (P-bodies; PB) (8,9). P-bodies are one of the most well understood types of RNA granules, and are associated with a variety of diseases, including cancer (10,11). Certain PB-associated proteins have been implicated in cancer cell physiology and may be involved in tumorigenesis (10-14). The effects of the expression of DCP1a on the prognosis of colorectal carcinoma remain unclear.

In the present study, DCP1a mRNA expression levels, protein levels and immunohistochemical staining in carcinoma, and non-carcinoma tissues of 75 patients were examined. In addition, the association between the expression of DCP1a and clinicopathological parameters was investigated. It was identified that high levels of expression of DCP1a may result in deeper invasion, higher lymph node metastasis rate and later TNM stage, which results in lower survival rates following surgery.

## Materials and methods

**Patients and tissues samples.** A cohort of 118 patients with CRC who underwent surgical resection at Union Hospital of Huazhong University of Science and Technology (Wuhan, China) between March 2010 and March 2011 was enrolled.

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**Key words:** mRNA-decapping enzyme 1a, colorectal carcinoma, survival rate, prognostic indicator

The classification of the 118 cases was performed according to the American Joint Committee on Cancer 2010 classification system (15-17). The enrolled patients had not received any previous form of preoperative therapy. Tissue samples, surgically resected from these patients, were confirmed by pathological analysis following hematoxylin and eosin staining. Non-carcinoma tissues were obtained 5 cm from the tumor sections. A total of 6 pieces of each specimen were obtained for examination. Of these pieces, 3 were immersed in formalin, and the others were stored in liquid nitrogen immediately until use. The final survival data were collected on March 30, 2016. The present study was approved by the Ethics Committee of Huazhong University of Science and Technology for Clinical Investigation (Wuhan, China). All patients provided written informed consent prior to enrollment.

*Levels of mRNAs evaluated by reverse transcription quantitative polymerase chain reaction (RT-qPCR).* In order to analysis the relative levels of mRNA expression, total RNA was collected from clinical specimens using the miRNeasy kit (Qiagen, Inc., Valencia, CA, USA) according to manufacturer's protocol. The primers for DCPIa were as follows: Forward, CACCCCGGTGCTAATCACTC and reverse, GCTCAACGGGATTGTGTAGGTT. The primers for GAPDH were as follows: Forward, AGACAGCCGCATCTTCTTGT and reverse, CTTGCCGTGGGTAGAGTCAT. Firstly, strand cDNA was synthesized from 1  $\mu$ g RNA using cDNA Reverse Transcription kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA) according to the manufacturer's protocol. The cDNA transcripts (20 ng) synthesized in the present study were used as the template for RT-qPCR using the SYBR-Green method (Bioline Reagents, Ltd., London, UK). The iCycleriQ Real-Time Detection system software v2.3 (Bio-Rad Laboratories, Inc.) was used to determine the level of mRNA. The thermocycling conditions were as follows: Initial activation step (95°C for 20 sec) followed by 40 cycles of 95°C for 6 sec and 60°C for 45 sec. The melting curve was obtained by an initial denaturation step (95°C for 20 sec) followed by a gradual heating from 60 to 95°C (ramp of 0.3°C). The data was analyzed by the Cq method and normalized using GAPDH expression in each sample (18). The primers pairs for the DCPIa and GAPDH were purchased from Sino Biological, Inc. (Beijing, China).

*Immunohistochemistry.* Tissue samples for immunohistochemical analysis were selected by a pathologist. Subsequent to being fixed in 4% formalin and embedded in paraffin for 2 h at 67°C, the tissue specimens were cut into consecutive 4- $\mu$ m thick sections. Following de-paraffinization with xylene (5 min, 3 times) and gradient rehydration (100% anhydrous ethanol, 10 min twice; 95% ethanol, 10 min twice; distilled water, 5 min twice) at room temperature, antigen retrieval steps were performed as follows: i) Citric acid buffer processing, where the slides were soaked in 10 mM of citrate buffer (pH 6.0), maintained at a boiling temperature (95-99°C) for 10 min and then cooled for 30 min at room temperature; ii) EDTA treatment, where the slides were soaked in 1 mM EDTA solution (pH 8.0) and maintained at a boiling temperature (95-99°C) for 15 min; iii) Tris-EDTA (TE) processing, where the slides were soaked in 10 mM TE/1 mM EDTA

solution (pH 9.0) and maintained at a boiling temperature (95-99°C) for 18 min, and then cooled for 30 min at room temperature; and iv) Pepsin processing, including digestion for 10 min at 37°C. Then the slides were blocked with goat serum (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) at 37°C for 10 min and incubated with polyclonal anti-DCPIa antibody (1:100 dilution; cat. no. ab47811; Abcam, Cambridge, MA, USA) at 4°C overnight. Then the slides were incubated with a secondary antibody (1:2,000; cat. no. ab191866; Abcam; anti-rabbit IgG VHH Single Domain antibody; horseradish peroxidase conjugated) at room temperature for 30 min. The slides were incubated in DAB, and stained with hematoxylin following incubation with a horseradish peroxidase-conjugated lectin at room temperature for 30 min. Then, the slides were washed with distilled water, dehydrated (95% ethanol, 10 sec twice; anhydrous ethanol, 10 sec twice; xylene, 10 sec twice) and mounted using neutral balsam. Then, the slides were observed under a light microscope at a magnification of x100, and 5 fields of view were selected for analysis. The data was observed by eye. The level of DCPIa expression was compared with the non-carcinoma tissues and designated as high expression when it was increased compared with that in the non-carcinoma tissue. The threshold value was dependent on the expression level in normal tissues.

*Western blotting.* Total protein was extracted from the tissues using radioimmunoprecipitation assay lysis buffer (Beyotime Institute of Biotechnology, Haimen, China). Following incubation with the buffer at 0-4°C for 30 min, the cellular debris was removed by centrifugation at 15,000 x g at 4°C for 30 min. Then, the supernatant was collected. A total of 50  $\mu$ g of each protein sample (the concentrations of the protein samples were obtained using the BCA method (19) and the BCA protein assay kit (Thermo Fisher Scientific, Inc.) was added to 5X loading buffer (Boster Biological Technology, Pleasanton, CA, USA; volume ratio, 4:1), and boiled (95-99°C) for 5 min. Following separation using 10% SDS-PAGE, the proteins were transferred to a polyvinylidene fluoride membrane by standard electroblotting procedures. The membranes were probed with anti-DCPIa antibody (1:100 dilution; cat. no. ab47811; Abcam; rabbit polyclonal for Dcpl1a) and anti-GAPDH antibody (1:200 dilution; cat. no. ab181602; Abcam; rabbit polyclonal for GAPDH) at 4°C overnight. Then, the membranes were incubated with a secondary antibody (1:2,000; cat. no. ab191866; Abcam; anti-rabbit IgG VHH Single Domain antibody; horseradish peroxidase conjugated) at room temperature for 1 h. The protein bands were detected by chemiluminescence using an ECL detector (Thermo Fisher Scientific, Inc.).

*Statistical analysis.* All the data were analyzed by SPSS software version 20.0 (IBM Corp., Armonk, NY, USA) and all the experiments were repeated three times, and the data are presented as mean  $\pm$  95% confidence interval. A Student's t-test was used to analyze the difference in DCPIa mRNA expression between the clinical carcinoma and non-carcinoma tissues. Analysis between DCPIa expression and clinicopathological parameters in colorectal carcinoma was performed using a  $\chi^2$  test. Cox's regression analysis was used to evaluate the clinicopathological characteristics with respect to carcinoma-specific survival. Survival curves were estimated by the

Table I. Association between DCP1a expression and clinicopathological parameters in colorectal carcinoma.

Clinicopathological parameters	DCP1a expression		P-value <sup>a</sup>
	Low (n=32)	High (n=86)	
Age, years (mean $\pm$ SD)	56.59 $\pm$ 11.34	57.34 $\pm$ 12.09	0.763
Sex			
Female	17	32	0.119
Male	15	54	
Tumor location			
Colon	18	51	0.765
Rectum	14	35	
Depth of invasion			
T1/T2	15	19	0.008
T3/ T4	17	67	
Lymph node metastasis			
Negative	23	32	0.001
Positive	9	54	
TNM stages			
I/II	22	29	0.001
III/IV	10	57	

<sup>a</sup>Differences between the two groups were evaluated by  $\chi^2$  test. TNM, tumor node metastasis; SD, standard deviation; DCP1a, mRNA-decapping enzyme 1a.

Kaplan-Meier method and differences in survival rates were detected by the log-rank test.  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**Relative DCP1a mRNA expression level in colorectal carcinoma and non-carcinoma tissues.** A RT-qPCR assay was performed to evaluate the relative DCP1a mRNA expression levels in the clinical carcinoma and non-carcinoma tissue specimens of 118 CRC cases. The result was analyzed by Student's t-test, and as indicated in Fig. 1 the expression level of DCP1a was significantly increased in the carcinoma specimens compared with the non-carcinoma tissues ( $P = 0.0024$ ). This indicates that DCP1a may be a diagnostic biomarker for patients with colorectal carcinoma.

**DCP1a protein expression in CRC and non-carcinoma tissues.** Immunohistochemical staining was performed to investigate the expression levels of DCP1a in the tissues, and the results are presented in Fig. 2A. It was identified that DCP1a protein exhibited a visibly higher expression in carcinoma tissues compared with the non-carcinoma tissues. In addition, the results of 3 representative samples from the western blot analysis are presented, demonstrating the protein levels of DCP1a in the carcinoma and non-carcinoma tissues (Fig. 2B). The results of western blot analysis indicated that the DCP1a protein level in the CRC samples was markedly increased compared with non-carcinoma tissues, with the exception of 32 cases. The underlying mechanism requires additional exploration.

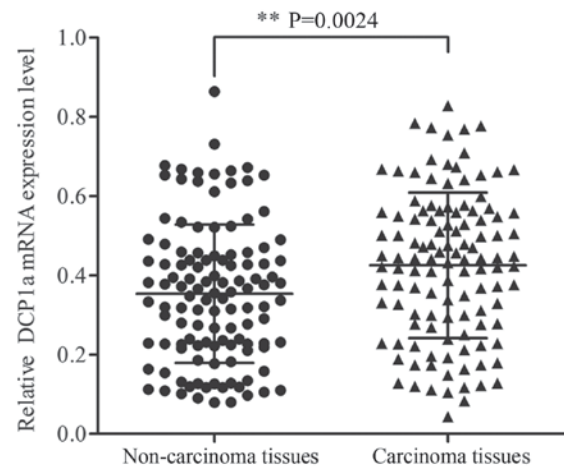


Figure 1. Relative mRNA level of DCP1a in colorectal carcinoma and non-carcinoma tissues by reverse transcription quantitative polymerase chain reaction, and analyzed by Student's t-test. The relative level of DCP1a mRNA identified in carcinoma tissues was significantly increased compared with that in non-carcinoma tissues. Experiments were repeated three times ( $^{**}P = 0.0024$ ). DCP1a, mRNA-decapping enzyme 1a.

**Association between DCP1a expression and clinicopathological parameters in colorectal carcinoma.** According to the level of DCP1a expression compared with the non-carcinoma tissues, the cases were divided into low expression ( $n = 32$ ) and high expression ( $n = 86$ ) groups; levels of DCP1a expression higher compared with the non-carcinoma tissues were categorized as high expression. The clinicopathological parameters were collected and are summarized in Table I, and the differences between the two groups were evaluated using the  $\chi^2$  test. No statistically significant

Table II. Cox regression analysis of clinicopathological characteristics and carcinoma-specific survival.

Variable	Hazard ratio	95% confidence interval	P-value
DCP1a level (high vs. low)	4.042	1.597-10.226	0.003
Sex (female vs. male)	0.844	0.466-1.531	0.577
Location (colon vs. rectum)	1.333	0.752-2.366	0.325
Depth of invasion (T3/T4 vs. T1/T2)	2.428	1.133-5.202	0.023
Lymph node metastasis (yes vs. no)	4.391	2.178-8.855	<0.001
TNM stage (III/IV vs. I/II)	5.275	2.458-11.324	<0.001

DCP1a, mRNA-decapping enzyme 1a; TNM, tumor node metastasis.

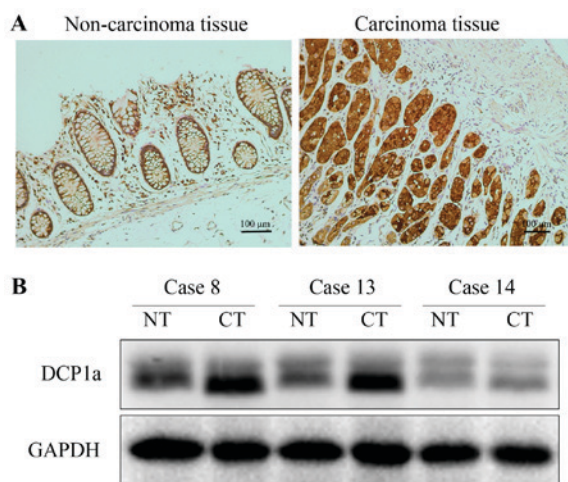


Figure 2. DCP1a protein expression in colorectal carcinoma and non-carcinoma tissues. (A) Immunohistochemical staining indicated that the level of DCP1a protein was visibly increased in carcinoma tissues compared with the non-carcinoma tissues (magnification, x100). (B) Western blotting was performed to detect the levels of DCP1a, and representative gels of 3 patients are presented. All the experiments were repeated 3 times. DCP1a, mRNA-decapping enzyme 1a; NT, normal tissue; CT, carcinoma tissue.

association was identified between the expression of DCP1a, and age, sex and tumor location. Conversely, an association was identified between a high expression of DCP1a in patients with CRC and deeper levels of invasion ( $P=0.008$ ), a higher rate of lymph node metastasis ( $P=0.001$ ), and later TNM stage ( $P=0.001$ ). In conclusion, the results obtained indicate that the expression of DCP1a may serve a role in the development and progression of colorectal carcinoma.

*Survival rate with the expression of DCP1a and clinicopathological characteristics with respect to carcinoma-specific survival.* As the survival curve suggests in Fig. 3, the low DCP1a-expression group exhibited an increased survival rate at the end of follow-up in comparison with the high-expression group ( $P=0.001$ ). Additional analysis of the clinicopathological characteristics with respect to carcinoma-specific survival is summarized in Table II. Cox regression analysis revealed that the DCP1a expression ( $P=0.003$ ), depth of invasion ( $P=0.023$ ), lymph node metastasis ( $P<0.001$ ) and TNM stage ( $P<0.001$ ) had a significant effect on survival rate. In summary, the high expression of DCP1a may lead to poorer prognosis in the patients with CRC compared with patients with low DCP1a

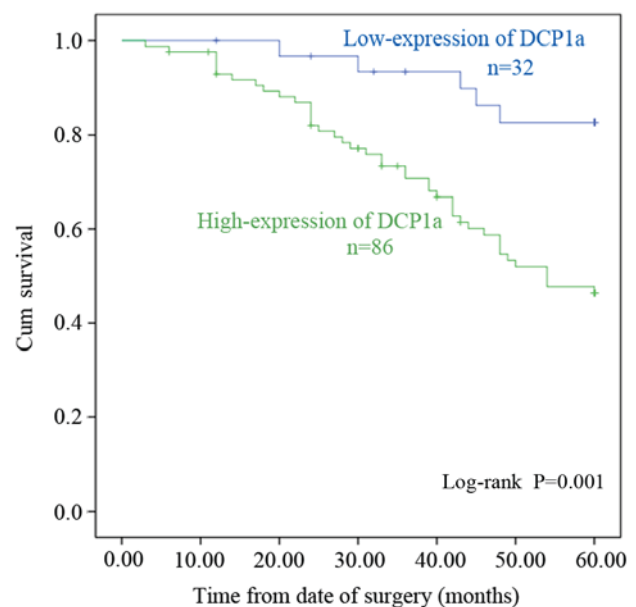


Figure 3. Overall survival of 118 patients with colorectal carcinoma in association with DCP1a expression levels. All patients with CRC were divided into high expression ( $n=86$ ) and low expression ( $n=32$ ) groups. The levels of DCP1a expression were compared with the normal tissues. Survival curves were analyzed by log-rank tests. The survival rate in the high-expression group was significantly decreased in comparison with that in the low-expression group ( $P=0.001$ ).

expression. These data suggest that DCP1a may be a useful prognostic indicator in clinical practice.

## Discussion

CRC is one of the most frequently diagnosed types of cancer and cause of mortality worldwide (2,20). Despite the improved diagnostic methods and therapies, the 5-year survival rate for CRC remains unsatisfactory (20,21). Thus, the identification of novel diagnosis and treatment methods is required. The roles of mRNAs in eukaryotic cells have been well characterized; they are important in regulating gene expression and may be promising in cancer therapies (22,23). Notably, eukaryotic cellular mRNAs possess distinctive sections at their 5'- and 3'-ends. It was also identified that the 5'-7-methylguanosine cap structure and the 3'-terminal poly (A) tract perform a variety of functions in the synthesis, translation and degradation of mRNA (24). As the removal of the cap structure is an



essential step in mRNA degradation in eukaryotes, decapping enzymes are significant in the process.

P-bodies are cytoplasmic structures involved in mRNA degradation (9). The key factors that promote the assembly of mRNPs into P-bodies include the activation of decapping and 5' to 3' decay of mRNA, which makes analyzing the function of DCP1a important (25,26). Several P-bodies-associated proteins, including cap-binding protein eIF4E and eIF5A, are involved in tumor genesis and progression (10,27-29). As revealed in our previous study, single nucleotide polymorphisms in DCP1a were associated with an increased risk of melanoma-specific mortality (30). However, whether the expression of DCP1a is associated with CRC remains unclear.

In the experiments of the present study, it was identified that the levels of DCP1a mRNA and protein exhibited increased levels of expression in carcinoma tissues compared with non-carcinoma tissues. The mechanism underlying this phenomenon requires additional study. In order to analyze the association between the expression of DCP1a with clinicopathological characteristics, follow-up investigation was performed. Although no significant association between the age, sex and tumor location and the expression of DCP1a was observed, high levels of DCP1a expression were associated with deeper invasion, higher rate of lymph node metastasis, and later TNM stage. Concomitant with this result, according to the follow-up data it was also identified that the expression of DCP1a had an effect on survival rate. The Cox regression analysis additionally confirmed that DCP1a was an independent factor affecting the survival time of patients with CRC following surgery. Taken together, it was elucidated that high DCP1a expression levels were associated with poor prognosis, and may serve an important role in carcinogenesis and progression. To the best of our knowledge, this is the first study to demonstrate the association between the expression of DCP1a and the clinical features of CRC.

In conclusion, DCP1a may have high expression in CRC tissues and be associated with poor prognosis. This feature suggests that DCP1a may be a sensitive diagnostic marker or prognostic indicator, which may improve patient assessment and the generation of therapy plans. As DCP1a is important in P-body formation and regulation, it may participate in regulating P-bodies-associated proteins, which may be beneficial for carcinogenesis. The findings of this study may assist with developing novel therapeutic strategies to increase the benefits of treatments in patients with CRC.

#### Acknowledgements

Not applicable.

#### Funding

The present study was funded by the National Natural Science Foundation of China (grant no. 81600401) and the Natural Science Foundation of Hubei Province, China (grant no. 2017CFB474).

#### Availability of data and materials

All data generated or analyzed during this study are included in this published article.

#### Authors' contributions

KT and WZ designed the study and performed all surgery. CW and TR wrote and revised the manuscript, CW and WL performed the experiments and acquired data. XZ and TR analyzed the data.

#### Ethical approval and consent to participate

The present study was approved by the Ethics Committee of Huazhong University of Science and Technology for Clinical Investigation. The patients provided written informed consent prior to enrollment.

#### Consent for publication

All patients provided written informed consent for the publication of their data.

#### Competing interests

The authors declare that they have no competing interests.

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