

## Original Article

# Expression and clinical significances of Beclin1, LC3 and mTOR in colorectal cancer

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**Abstract:** Autophagy is related to cancer and other diseases, and compromised autophagy could promote chromosome instability associated with carcinogenesis and tumor progression. The role of autophagy in the growth and metastasis of colorectal cancer (CRC) remains poorly understood. Beclin1 mediates autophagic initiation, and LC3 is a specific marker for autophagy. Inactivation of mTOR caused by cellular hypoxia or energy deficiency induces autophagic activity. This study aims to examine the expression and clinical significance of these proteins in CRC. Immunohistochemistry results showed that the positive expression rates of Beclin1, LC3, and mTOR in cancer tissues were 90.50%, 87.19%, and 46.28%, respectively, which were higher than those in adjacent tissues ( $P < 0.05$ ). Differentiation degree and lymph node metastasis were associated with LC3 overexpression ( $P < 0.05$ ) but not with Beclin1 ( $P > 0.05$ ). Lymph node metastasis was also related to mTOR. Spearman analysis results showed that LC3 expression was positively correlated with Beclin1 but negatively correlated with mTOR ( $r = 0.593$  and  $-0.165$ , respectively;  $P < 0.01$ ). Beclin1 expression was also not associated with mTOR ( $P > 0.05$ ). Survival analysis further indicated that LC3, mTOR, and lymph node metastasis were independent prognostic factors in CRC. Real-time PCR results and Western blot indicated that Beclin1, LC3, and mTOR expression in CRC was significantly higher than that in adjacent tissues ( $P < 0.01$ ). The aberrant protein expression may be associated with the development and progression of CRC. The LC3 and mTOR genes must be simultaneously detected to evaluate progression and prognosis of CRC.

**Keywords:** Colorectal cancer, Beclin1, LC3, mTOR, prognosis

## Introduction

Colorectal cancer (CRC) is one of the most prevalent malignant tumors of the digestive system. The incidence and mortality of CRC continuously increase, and the age at onset shifts toward younger ages. Previous studies indicated that tumorigenesis resulted from infinite proliferation and apoptosis resistance of normal cells caused by gene mutation [1]. As an apoptosis-like biological phenomenon, autophagy has gained extensive attention. Autophagy is a highly conserved process of cellular self-destruction, in which cytoplasmic components, such as cytosolic proteins and organelles, are enclosed to form double-membrane autophagosomes; these vesicles can degrade the sequestered intra-autophagosomal components by using lysosome hydrolases [2]. Autophagy is related to cancer and other diseases [3], but its role in the growth and metastasis of CRC remains poorly understood.

Autophagy is a complex metabolism process regulated by multiple autophagy-related genes. Beclin1 (homolog of yeast Atg6 in humans) is the first identified mammalian autophagy-mediated gene involved in autophagic initiation; this protein results from allele deletion on chromosome 17q21 [4-6]. Beclin1 is a multifunctional protein that plays a significant role in cell differentiation, apoptosis, and autophagy. Upregulation of Beclin1, but not its basal levels, induces cell autophagy and differentiation. By contrast, knockdown of Beclin1 weakens these phenomena but apoptosis remains activated [7, 8]. Given the importance of this protein, the Beclin1-dependent signaling pathway has been the research focus in cancer prevention and targeted tumor therapy.

Microtubule-associated protein 1A/1B-light chain 3 (LC3), the homolog of yeast Atg8, is ubiquitously distributed and essential to the formation of autophagosomes in mammalian

## Beclin1 and LC3 and mTOR in colorectal cancer

**Table 1.** Correlation between Beclin1, LC3 and mTOR expression and clinicopathologic parameters in 202 cases of CRC patients

Characteristics	n	Beclin1		LC3		mTOR	
		+	P	+	P	+	P
Gender							
Male	127	117	0.364	113	0.382	63	0.656
Female	115	102		98		49	
Age (years)							
≤60	103	92	0.591	91	0.642	52	0.259
> 60	139	127		120		60	
Tumor size (cm)							
≤5	108	98	0.907	91	0.221	57	0.069
> 5	134	121		120		55	
Tumor location							
Colon	96	89	0.341	84	0.907	49	0.228
Rectum	146	130		127		63	
Differentiation degree							
High	67	57	0.199	52	0.021	28	0.278
Moderate	127	118		116		57	
Low	48	44		43		27	
TNM stage							
I+II	204	186	0.857	178	0.666	91	0.151
III+IV	38	33		33		21	
Lymph node metastasis							
Negative	133	119	0.549	107	0.000	47	0.000
Positive	109	100		104		63	

cells. During the formation, cytosolic LC3 (LC3-I) is transformed into membrane-bound LC3-II by conjugating to phosphatidylethanolamine (PE); this process is catalyzed by the E1-like enzyme Atg7 and the E2-like enzyme Atg3 [9-11]. The intra-autophagosomal LC3-I is degraded by lysosomal hydrolases during the fusion of au-tophosomes with lysosomes. Autophagic in-duction by starvation stimulates the conversion of LC3-I to LC3-II and upregulates LC3 expression; therefore, this protein is used as a specific marker for autophagy [12, 13].

Mammalian target of rapamycin (mTOR) is a member of the phosphatidylinositol-3-kinase family and is associated with the proliferation, survival, invasion, and metastasis of CRC cells [14]. This protein combines the upstream signals from insulin, growth factors, and mitogens and functions as an important sensor of cellular nutrients and energy. Previous studies showed that mTOR promotes protein synthesis, cell cycle, and angiogenesis and inhibits apoptosis and autophagy of colon tumors [15].

Cellular hypoxia or energy deficiency could lead to inactivation of mTOR and thus promotes autophagy [16].

In this study, we examined the status of autophagy-related genes, namely, Beclin1, LC3, and mTOR, through immunohistochemistry (IHC), Western blot, and real-time PCR. A total of 202 formalin-fixed paraffin-embedded specimens and 40 fresh frozen samples were obtained from patients with CRC. The relationship of Beclin1, LC3, and mTOR with clinicopathological characteristics was investigated. The correlation of Beclin1, LC3, and mTOR expression with CRC prognosis was also evaluated. To our knowledge, this study is the first to investigate the expression of Beclin1, LC3, mTOR and their relationship to the prognosis of patients with CRC.

### Materials and methods

#### *Patients and tissue specimens*

Formalin-fixed and paraffin-embedded CRC specimens and adjacent noncancerous tissues

were obtained from the archives of the Department of Pathology, Binzhou Medical University Hospital, China. The study group consisted of 242 patients with CRC who were admitted to our hospital between January 2004 and July 2008. They underwent curative resection at the Department of Gastroenterological Surgery, Binzhou Medical University Hospital, China. None of the patients underwent radiotherapy and chemotherapy prior to sample collection. Tumors were classified according to the American Joint Committee on Cancer guidelines and the criteria defined by the WHO International Histological Classification of Tumors [17]. The clinicopathological features detailed in **Table 1** included age, sex, tumor size, tumor position, level of tumor differentiation, and lymph node metastasis. Follow-up period was defined as the interval from the date of operation to the date of death or the last follow-up. A total of 202 patients were observed until July 2012, and the follow-up duration ranged from 10 to 89 months (median, 65.5 months). Fresh-frozen tumor tissues and the surrounding tissues (separated by > 5 cm) were used for protein preparation and RNA extraction; these tissues were collected from 40 patients with CRC who underwent curative resection between 2011 and 2012. Tumor specimens were obtained at surgery and stored in liquid nitrogen. The surrounding tissues were considered as the control tissue and were verified by the pathologist. The research was approved by the Ethics Committee of Binzhou Medical University Hospital, and all patients had signed an in-formed consent.

### *IHC*

The expression of Beclin1, LC3, and mTOR in primary tumors was detected through IHC and then compared with that in adjacent noncancerous tissues. Formalin-fixed paraffin-embedded archived tissues were cut into 4- $\mu$ m sections, dewaxed, rehydrated, and blocked with hydrogen peroxide. The sections were microwave heated with 10 mM citrate buffer (pH 6.0) for antigen retrieval antigens. The sections were then incubated with rabbit polyclonal antibody against human Beclin1 (Abcam, ab55878, Cambridge, UK), LC3 (Abcam, ab48394, Cambridge, UK), and mTOR (Abcam, ab2732, Cambridge, UK) at dilutions of 1:200, 1:50, and 1:200, respectively, overnight at 4°C. The sec-

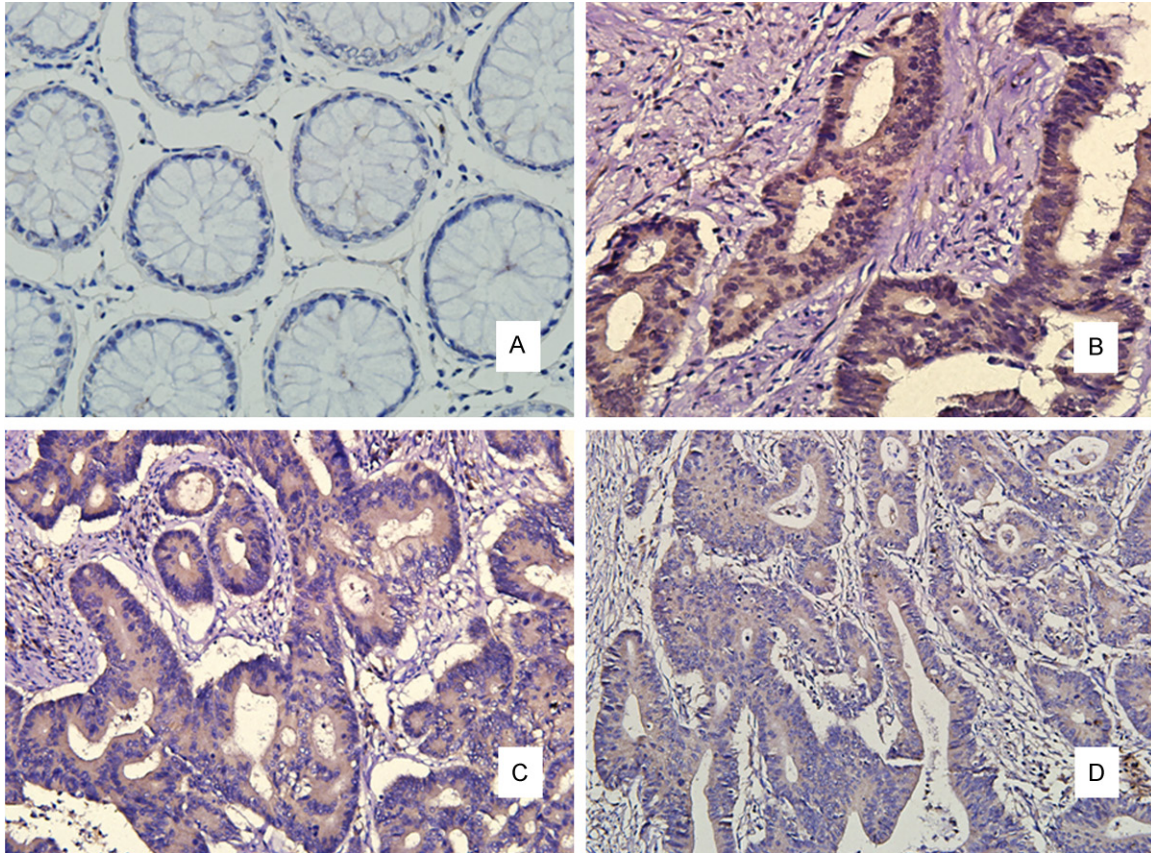
tions were subsequently incubated with horseradish peroxidase-labeled secondary antibody for 30 min. The sections were developed with diaminobenzidine tetrahydrochloride and counterstained with hematoxylin. IHC analysis was performed within 7 d from the preparation to prevent antigen degradation. A positive control was supplied by Abcam, and negative controls were prepared by replacing the primary antibody with PBS.

### *IHC evaluation*

The immunoreactivity of Beclin1, LC3, and mTOR was evaluated according to the intensity and percentage of positively stained cells. Immunostaining intensity was rated as follows: 0, negative; 1, weak; and 2, strong. The percentage of positively stained cells was graded as follows: grade 0, 0%-5%; grade 1, 6%-25%; grade 2, 26%-50%; grade 3, 51%-75%; and grade 4, 76%-100%. Immunoreactive score was calculated by adding the score of staining intensity and the percentage score of positively stained cells (0-6). Tumors with an immunoreactive score of 0-3 were designated as negative, and those with 4-6 were classified as positive. Immunostaining was evaluated twice by two pathologists who were blinded to patient outcomes and other clinical findings.

### *Western blot analysis*

Tissue samples were homogenized in SDS buffer containing the protease inhibitor PMSF. The homogenates were incubated on ice for 20 min and then centrifuged at 12,000 rpm for 30 min at 4°C. Supernatant was collected and added with a similar volume of 2 $\times$  SDS buffer. The mixture was boiled for 10 min and preserved at -20°C. The protein extracts (50  $\mu$ g) were separated through SDS-PAGE and then transferred onto polyvinylidene difluoride membrane (Millipore, USA). The membranes were blocked with 5% nonfat milk in Tris-buffered saline containing 0.1% Tween-20 at room temperature for 90 min. The membranes were then immunoblotted for Beclin1 (3  $\mu$ g/mL, Abcam, ab55878, Cambridge, UK), LC3 (2  $\mu$ g/mL, Abcam, ab48394, Cambridge, UK), mTOR (2  $\mu$ g/mL, Abcam, ab2732, Cambridge, UK), and actin (0.5  $\mu$ g/mL, Abcam, ab3280, Cambridge, UK). Pro-teins bands were detected with secondary antibodies conjugated to horseradish peroxi-



**Figure 1.** Immunohistochemistry analysis was used to measure the expression of Beclin1, LC3 and mTOR in colorectal cancer and normal colorectal mucosa tissues. Normal colorectal mucosa tissues show no immunoreactivity for Beclin1, LC3 and mTOR (A). Colorectal cancer tissues show immunoreactivity for Beclin1 (B), LC3 (C) and mTOR (D) in the cytoplasm. Magnification: (A-D) ×400.

dase (1:5000, Abcam, UK) and visualized with enhanced chemiluminescence reagents. Each band was quantified through densitometry, and results were presented as the relative expression of each protein from different samples.

#### *RNA collection, cDNA synthesis, and real-time PCR analysis*

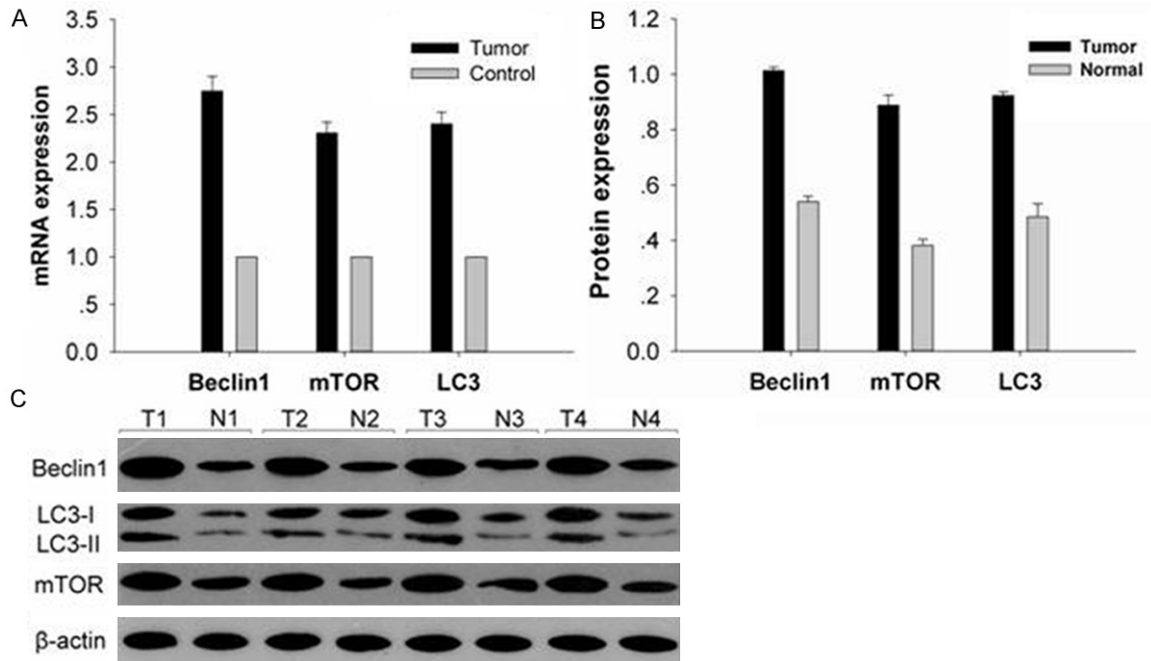
Total RNA was extracted from fresh-frozen tumor specimens and the corresponding non-cancerous tissues by using Trizol reagent (Invitrogen, USA). The amount of RNA was determined with the absorbance at 260 nm, and its purity was estimated using the ratio of the absorbance at A260/280. The total RNA was reverse transcribed using Prime Script RT reagent kit (Takara, DRR037A, China). Real-time PCR reactions were performed on the CF-X96TM real-time PCR detection system C1000 (Applied Biosystems, USA). For SYBR GREEN I-based real-time PCR reactions, each 25  $\mu$ L of

the reaction mixture contained 2  $\mu$ L of primer pairs, 2  $\mu$ L of cDNA, 12.5  $\mu$ L of SYBR Premix Ex TaqII, and ddH<sub>2</sub>O to obtain a final volume of 25  $\mu$ L. The primers used were as follows: Beclin1: Fwd-5'-ATGCAGGTGAGCTTCGTGTG-3', Rev-5'-CTGGGCTGTGGTAAGTAATGGA-3'; and LC3: Fwd-5'-AAACGCATTTGCCATCACA-3', Rev-5'-GGACCTTCAGCAGTTTACAGTCAG-3'.

#### *Statistical analysis*

Statistical analysis was performed with SPSS15.0 software (SPSS Inc., Chicago, IL, USA). Pearson's Chi-square test was used to evaluate the correlation of Beclin1, LC3, and mTOR expression with clinicopathological parameters of patients with CRC. Survival curves were calculated through Kaplan-Meier method and compared using log-rank test. The Cox proportional hazards regression model was used to determine the effects of clinicopathological variables and the expression of Beclin1, LC3,

## Beclin1 and LC3 and mTOR in colorectal cancer



**Figure 2.** Expression of Beclin1, LC3 and mTOR in CRC tissues and counterpart normal colorectal mucosa tissues. 40 cases CRC patients were detected by Real-time PCR (A) and Western blot (B). The data were shown as means  $\pm$  SE. Significant higher expression of Beclin1 ( $P < 0.01$  for mRNA level,  $P < 0.05$  for protein level.), LC3 ( $P < 0.01$  for mRNA level,  $P < 0.05$  for protein level.) and mTOR ( $P < 0.01$  for mRNA level,  $P < 0.05$  for protein level.) has been found in CRC tissues compared to counterpart normal colorectal mucosa tissues. (C) Proteins extracted from four matched pairs of representative colorectal cancer tissues (T1, 3, 5, and 7) and normal colorectal mucosa tissues (N2, 4, 6, and 8) were subjected to Western blot analysis. The expression of Beclin1, LC3 and mTOR were increased in colorectal cancers compared to counterpart normal colorectal mucosa tissues.

and mTOR on survival. Differences were considered significant if  $P$  value was lower than 0.05.

### Results

#### *High mRNA expression of Beclin1, LC3, and mTOR in CRC tissues*

To determine the expression of Beclin1, LC3, and mTOR between CRC and counterpart normal tissues, we detected their mRNA levels by using 40 fresh-frozen samples through real-time PCR. **Figure 2A** shows that the mRNA levels of Beclin1, LC3, and mTOR increased in CRC tissues; these levels were  $2.747 \pm 0.155$ ,  $2.412 \pm 0.121$ , and  $2.305 \pm 0.113$  times higher than those in the counterpart normal tissues, respectively ( $P < 0.05$ ).

#### *Increased protein expression of Beclin1, LC3, and mTOR in CRC tissues*

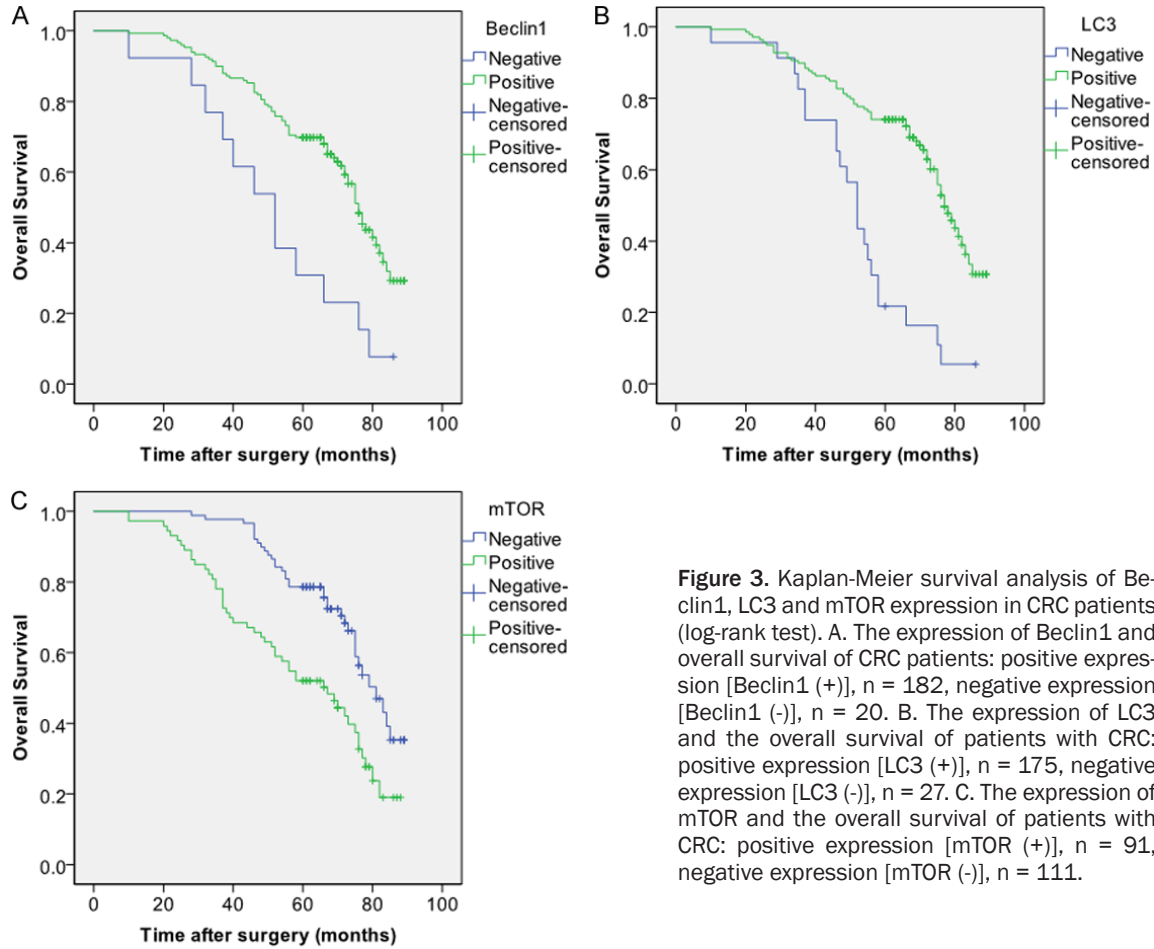
The protein levels of Beclin1, LC3, and mTOR in CRC and counterpart normal tissues were determined using Western blot analysis. The expression of these proteins was detected

using 40 fresh-frozen samples and then analyzed with Quantity One software. The relative expression levels of Beclin1, LC3, and mTOR proteins in CRC tissues were  $1.014 \pm 0.012$ ,  $0.923 \pm 0.014$ , and  $0.889 \pm 0.037$ , respectively ( $P < 0.05$ , **Figure 2B**); in the counterpart normal CRC tissues, these levels were  $0.485 \pm 0.047$ ,  $0.540 \pm 0.026$ , and  $0.382 \pm 0.023$ , respectively ( $P < 0.05$ , **Figure 2C**).

#### *Expression and correlation of Beclin1, LC3, and mTOR in CRC tissues*

The immunostaining of the proteins was observed in the cytoplasm of cancer cells, whereas no or very weak immunostaining was observed in normal epithelial cells. The immunostaining of Beclin1, LC3, and mTOR were 90.50%, 87.19%, and 46.28% in cancer cells, respectively, and 47.69%, 41.54%, and 23.07% in normal epithelial cells, respectively. The positive expression rates of Beclin1 (**Figure 1B**), LC3 (**Figure 1C**), and mTOR (**Figure 1D**) in CRC were significantly higher than those in the counterpart normal epithelial tissues ( $P < 0.01$ ,

## Beclin1 and LC3 and mTOR in colorectal cancer



**Figure 3.** Kaplan-Meier survival analysis of Beclin1, LC3 and mTOR expression in CRC patients (log-rank test). A. The expression of Beclin1 and overall survival of CRC patients: positive expression [Beclin1 (+)], n = 182, negative expression [Beclin1 (-)], n = 20. B. The expression of LC3 and the overall survival of patients with CRC: positive expression [LC3 (+)], n = 175, negative expression [LC3 (-)], n = 27. C. The expression of mTOR and the overall survival of patients with CRC: positive expression [mTOR (+)], n = 91, negative expression [mTOR (-)], n = 111.

**Figure 1A).** Spearman test showed that Beclin1 was positively associated with LC3 in CRC with a correlation coefficient of 0.593 ( $P < 0.01$ ), whereas mTOR was inversely correlated with LC3 with a correlation coefficient of -0.165 ( $P < 0.01$ ). No significant correlation was found between Beclin1 and mTOR expression in CRC.

### Association of Beclin1, LC3, and mTOR expression with clinicopathological parameters of CRC

We used IHC to analyze the relationship of Beclin1, LC3, and mTOR protein expression with clinicopathological features of CRC. We found that LC3 expression was significantly correlated with clinical features. As shown in **Table 1**, the overexpression of LC3 was associated with less cell differentiation ( $r = 0.075$ ,  $P < 0.05$ ). The expression of mTOR was also associated with cell differentiation ( $r = 0.284$ ,  $P < 0.05$ ) and lymph node metastasis ( $r = 0.530$ ,  $P < 0.05$ ). The expression of Beclin1 was not sig-

nificantly associated with clinicopathological parameters, including patient age, gender, tumor size, primary site, cell differentiation, and lymph node metastasis.

### Kaplan-Meier analysis

The expression of Beclin1, LC3, and mTOR was also evaluated to elucidate the correlation between autophagy and survival time after radical resection. An intact follow-up in 202 patients with CRC was performed, and the median survival time was 60.5 months. As shown in **Figure 3**, Kaplan-Meier analysis revealed that Beclin1, LC3, mTOR, cell differentiation, and lymph node metastasis were prognostic parameters. The 5-year survival rates of patients with positive and negative Beclin1 expression were 61.2% and 35.8%, respectively ( $P < 0.01$ , **Figure 3A**). The 5-year survival rates of patients with positive and negative LC3 expression were 64.3% and 32.1%, respective-

## Beclin1 and LC3 and mTOR in colorectal cancer

**Table 2.** Cox regression model analysis of overall survival in 202 CRC patients

Parameters	B	SE	Wald	df	Exp (B)	95.0% CI	P
LC3	- 1.015	0.212	22.871	1	0.362	0.239 ~ 0.549	0.000
mTOR	0.804	0.221	13.174	1	2.234	1.447 ~ 3.449	0.000
Lymphnode metastasis	0.772	0.194	15.773	1	2.165	1.479 ~ 3.170	0.000

ly ( $P < 0.01$ , **Figure 3B**). The 5-year survival rates of patients with high and low mTOR expression were 52.1% and 78.7%, respectively ( $P < 0.01$ , **Figure 3C**).

### Cox proportional hazards regression model analysis

As shown in **Table 2**, the Cox model revealed that LC3, mTOR, and lymph node metastasis were independent prognostic parameters for patients with CRC. LC3 was associated with long survival (-1.197 for the partial regression coefficient, 0.302 for relative risk, 95.0% CI for relative risk = 0.183-0.500). The expression of mTOR was inversely correlated with survival time (0.804 for the partial regression coefficient, 2.234 for relative risk, 95.0% CI for relative risk = 1.447-3.449). Lymph node metastases were significantly associated with short survival (0.545 for the partial regression coefficient, 1.725 for relative risk ( $P < 0.01$ ), 95% CI for relative risk = 1.099-2.708). These findings confirmed that these parameters can be used to evaluate prognosis in CRC. LC3 was related to positive prognosis, whereas high mTOR expression and lymph node metastasis were inversely correlated with the prognosis of patients with CRC. Moreover, survival was not related to the remaining clinicopathological parameters, such as age, sex, size, and site of primary mass, although female patients exhibited shorter survival ( $P = 0.072$ ).

### Discussion

In the present study, we investigated the expression and significance of three autophagy-related proteins, namely, Beclin1, LC3, and mTOR, in the tumorigenesis and development of CRC. By using IHC, we observed that these proteins were mainly located in the cell cytoplasm and their expression was significantly higher in CRC tissues than that in the counterpart normal tissues. The expression of LC3 was also positively correlated with Beclin1 but negatively correlated with mTOR in CRC tissues. We also

analyzed the association between protein expression and clinicopathological characteristics, including patient age, gender, tumor size, primary site, cell differentiation, and lymph node metastasis. The expression of LC3 was positively associated with cell differentiation, and mTOR was positively associated with cell differentiation and lymph node metastasis. The expression of Beclin1 was not significantly correlated with clinicopathological characteristics.

Compromised autophagy could promote chromosome instability associated with carcinogenesis and tumor progression [18]. Beclin1, the mammalian homolog of yeast Atg6, has been investigated in breast, melanoma, and ovarian cancers. Won et al. [5] reported that the expression of Beclin1 decreases in human breast cancer cells compared with that in normal ductal epithelium through IHC. Liang [19] observed that the expression of Beclin1 decreases in breast cancer cells compared with that in normal epithelial cells from the breast. In this study, the Beclin1 gene was deleted mono-allelically in human MCF7 breast cancer cell lines and its re-expression restores autophagy and suppresses tumorigenesis. Clelia et al. [20] also demonstrated that Beclin1 expression decreases in human melanoma by using IHC, Western blot, and real-time PCR. The expression of Beclin1 also decreases in malignant epithelial ovarian cancers compared with that in benign and borderline ovarian tumors [21]. In our present study, the expression of Beclin1 significantly increased in CRC tissues compared with that in the counterpart noncancerous tissues in the mRNA and proteins levels. The overexpression of Beclin1 could be interpreted in several ways. During the development and progression of cancers, cancer cells are often exposed to metabolic stress, such as insufficient nutrients or oxygen supplies, because of the high proliferation rate and insufficient vascularization. The Beclin1 expression in CRC cells may induce autophagy, which may confer survival by promoting vascularization or

protecting cancer cells from therapeutic intervention.

LC3 exists as a cytosolic form of LC3 (LC3-I) and a membrane-bound form of LC3II in mammalian cells. During autophagy, LC3-I is conjugated to PE to form LC3-II. The conversion of LC3-I to LC3-II is associated with autophagic activity and can be detected with Western blot. Consequently, detecting LC3 through immunoblotting is a reliable method to monitor autophagy [22, 23]. Jiang et al. [24] further demonstrated that the expression of LC3 decreases in human lung cancer tissues compared with that in the counterpart normal tissues, but this expression is not significantly correlated with clinicopathological characteristics. In our present study, the expression of LC3 significantly increased in CRC tissues compared with that in the counterpart noncancerous tissues in the mRNA and protein levels. The expression of LC3 was significantly associated with cell differentiation and its distinct expression patterns in CRC and lung cancer may be associated with tumor type [25]. Our study indicated that autophagic activity or the potential for autophagy increased in cancer cells and may be related to tumorigenesis of CRC. Moreover, the significant correlation between the expression of LC3 and cell differentiation suggested that cell malignancy may result from increased autophagy.

The kinase of mTOR is the major target in the mammalian autophagy signaling pathway, which regulates cell transcription, translation, and cytoskeletal organization. The expression of mTOR increases in prostate, liver, and head and neck cancers [26-28]. In our present study, the expression of mTOR increased in CRC and was associated with cell differentiation and lymph node metastasis. Hence, the overexpression of mTOR may be associated with the development, invasion, and metastasis of CRC.

Beclin1 is an autophagy-related gene that can activate autophagy, whereas mTOR is a negative target of autophagy. In our study, the expression of LC3, a specific marker for autophagy, was positively correlated with Beclin1 and negatively correlated with mTOR in CRC tissues. The positive expression of Beclin1, LC3, and mTOR was also significantly higher in CRC tissues than that in counterpart normal tissues. This finding suggested that autophagy may be correlated with the activation of Beclin1 in CRC.

mTOR plays a minor role only in inhibition of autophagy in CRC, but its high expression may play an important role in the proliferation and metastasis of tumor. Given that the regulatory mechanism of autophagy is complicated, further studies must investigate the mechanical regulation between mTOR and autophagy and the role of mTOR in CRC.

This study highlights the prognostic significance of mTOR and LC3 in CRC. Clinical studies revealed that the aberrant expression of Beclin1 is associated with poor prognosis in CRC and endometrial adenocarcinoma [19]. Giatromanolaki et al. [29] demonstrated that a relatively lower expression of LC3A in CRC is a marker of positive prognosis. However, in our present study, the high expression of LC3 was positively associated with long patient survival and the high expression of mTOR was positively associated with short patient survival. Hence, LC3 may act as a tumor suppressor and mTOR may act as a tumor promoter in CRC. Less cell differentiation and lymph node metastases were also associated with short 5-year survival. The role of autophagy in tumorigenesis and prognosis of CRC is possibly dependent on other parameters, including tumor stage, tissue type, and accompanying oncogene mutation. Future investigation must focus on the context-specific role of autophagy in CRC.

In conclusion, the overexpression of Beclin1, LC3, and mTOR may be associated with the development and progression of CRC. The high expression of LC3 and the low expression of mTOR were positively associated with survival of patients with CRC. Conversely, less cell differentiation and lymph node metastases were negatively associated with survival. Our findings suggested that the expression of LC3 and mTOR can be used as prognostic markers for survival of patients with CRC.

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### Disclosure of conflict of interest

None.

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## Beclin1 and LC3 and mTOR in colorectal cancer

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## Beclin1 and LC3 and mTOR in colorectal cancer

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