Meta-analysis of the autophagy-associated protein LC3 as a prognostic marker in colorectal cancer

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Abstract. Microtubule-associated protein 1 light chain 3 (LC3) is an autophagy-associated gene, which is involved in the progression of a number of human malignancies. Such as Breast Cancer, Liver Cancer, and Lung Cancer. However, the role of LC3 in colorectal cancer (CC) remains to be fully elucidated. Therefore, the prognostic role of LC3 expression in CC was evaluated in the present study, with an emphasis on the clinicopathology and prognosis. Expression of LC3 in CC was examined using PubMed, Cochrane Library, Excerpta Medica Database, China Knowledge Infrastructure and Wanfang Data. Newcastle-Ottawa scale was used to screen the literature quality, and RevMan 5.4 and STATA 14.0 were used for the meta-analysis. A total of 1,689 patients from 10 studies were included in the present meta-analysis. The findings of the present study suggested that increased LC3 expression levels were associated with histological grade [odds ratio (OR)=0.91, 95% confidence interval (CI) (0.47, 1.77), P<0.001] and TNM stage [OR=0.91, 95% CI (0.47, 1.77), P<0.001], but were not associated with sex [OR=1.14, 95% CI (0.90, 1.51)], age [OR=0.89, 95% CI (0.67, 1.20)], tumor size [OR=0.78, 95% CI (0.30, 2.34)], histological grade [OR=0.82, 95% CI (0.43, 1.95)] and lymph node metastasis [OR=2.05, 95% CI (1.19, 3.60)] in CC. In addition, the increased expression of LC3 was revealed to be a prognostic factor for the overall survival of patients with CC. In conclusion, the autophagy-associated protein LC3 may be a prognostic indicator of human CC.

Introduction

Colorectal cancer (CC) is the third most common malignant tumor globally and accounts for ~9.7% of all malignant tumors, with higher incidences in Europe, Australia and North America (1). Surgical resection combined with chemotherapy or radiotherapy remains the primary treatment for CC (2). However, due to the genetic differences between individuals, drug resistance remains an issue (3). For example, resistance to doxorubicin can occur due to decreased drug uptake or increased drug efflux through drug transporters present on the cell membrane. Moreover, cancer cells can develop mechanisms to detoxify and eliminate doxorubicin from the cellular environment. Previous studies have demonstrated that tumorigenesis is caused by gene mutations in cells Such as TP53, which results in an unrestricted cellular proliferation and resistance to apoptosis (4-6). Autophagy is an apoptosis-like biological phenomenon. In the initial stage of cancer, autophagy promotes the survival of normal cells and inhibits carcinogenesis by removing damaged organelles and DNA. In the advanced stage of cancer, autophagy provides sufficient nutrients for proliferation and metabolism of tumor cells and induces the survival of cancer cells, promote the metastasis of cancer cells to distant locations and increase their drug resistance (7). In addition, autophagy is a research target for cancer therapy by affecting cancer cells, stromal cells, and immune cells in the complex cancer microenvironment (8).

Autophagy, also known as type II programmed cell death, is a highly-conserved process of cellular destruction that transfers intracellular substances (including proteins, lipids and organelles) to lysosomes for degradation. The degraded intracellular materials are then released from the lysosomes and recycled in the cytosol (9). Autophagy is crucial in various types of cancer, including breast, lung, brain and CC (10). The effect of autophagy on cancer development may depend on the cancer type and the stage of cancer progression. In early stage cancer, autophagy is often thought to have a tumor suppressor effect. However, at a later stage, when the tumor microenvironment becomes more hostile, autophagy can turn to promote tumor growth and progression. The biological function of autophagy in cancer is complex and is likely dependent on type of tumor, stage and genetic context (11). The function of autophagy changes at different stages of cancer. In its early stages, autophagy may help suppress tumor growth by removing damaged cell components and inhibiting genomic instability. However, in later stages, autophagy can promote the survival of cancer cells under stressful conditions, such as nutrient deprivation or lack of oxygen, allowing them to adapt and

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grow. The process of autophagy can be both tumor-promoting and tumor-suppressing. Autophagy can suppress pathological processes, such as tumor metastasis, by acting as an intracellular quality control system. By removing damaged organelles, protecting against oxidative stress, inhibiting inflammation, and regulating cell death, autophagy acts as a defense mechanism that prevents or limits tumor metastasis. By contrast, autophagy may help tumors better adapt to adverse environments (12). For example, tumor cells can activate autophagy as a protective mechanism against various anti-cancer treatments, including chemotherapy and radiation therapy. Autophagy enables tumor cells to survive and recover from the induced stress, thereby promoting resistance to therapy. However, tumors can outgrow their blood supply, leading to areas of low oxygen levels (hypoxia). Hypoxia-induced autophagy enables tumor cells to adapt to this stressful condition by promoting cell survival and supporting angiogenesis, the formation of new blood vessels.

Autophagy is a complex metabolic process, which is regulated by autophagy-specific genes. Cleavage of light chain 3 (LC3)-I to LC3-II marks the beginning of autophagy (13). Subsequently, LC3-II binds to p62 (an adaptor protein) and promotes the autophagic degradation of ubiquitinated protein aggregates (14). LC3 and p62 are biomarkers that are commonly used for monitoring the levels of autophagy (15). LC3 is an indispensable component of autophagosomes (16). It contains the LC3A/B/C gene variants, with LC3B being most closely associated with autophagy (17). Previously, LC3 has been reported to serve a role in number of malignancies including the brain, colorectal and melanoma (8,18,19). However, previous studies have demonstrated opposing effects of the LC3 expression level on the overall survival (OS) of patients with different types of cancer (20,21). A previous study demonstrated a positive association between LC3 and hepatocellular carcinoma (HCC), and reported that LC3 was closely associated to the onset and progression of HCC (22). By contrast, another study demonstrated that LC3 is a protective factor in patients with CC (23). However, the relationship between the LC3 expression level and the clinicopathological traits of CC has not been reported. Therefore, the present meta-analysis investigated the relationship between the LC3 expression level and CC, and evaluated the prognostic effect of the LC3 expression level on CC.

Materials and methods

Literature examination strategy. PubMed, Cochrane Library, Excerpta Medica Database, China National Knowledge Infrastructure and Wanfang Data were used to examine the literature. The cut-off date for publication selection was February 2022. 'LC3' or 'microtubule-associated protein 1 light chain 3' and 'colorectal neoplasm' or 'colorectal tumor' or 'colorectal cancer' or 'colorectal carcinoma' were used as search terms.

Selection criteria. Inclusion criteria included: i) Cohort or case-control design; ii) patients were diagnosed according to pathological criteria; iii) literature provided sufficient clinicopathological and survival information to estimate the association between LC3 and CC; and iv) the full text report was issued in English or Chinese. Exclusion criteria included: i) Animal or cell experiments, case reports, reviews, letters, conference summaries or articles without full text; and ii) republished articles with analogous datasets or subjects.

Data extraction. The data extracted from the articles that were included in the present meta-analysis included the following information: Author, year of publication, country, sample size, patient characteristics (sex, age, tumor size, lymph node metastasis, histological grade and TNM stage), detection method and hazard ratio (HR) with 95% confidence interval (CI) for OS.

Quality evaluation. The Newcastle-Ottawa scale (NOS) was applied to evaluate the quality of the articles (24). A score of ≥6 denotes a high-quality study (Table I), scores of the 10 studies included in the present meta-analysis were all ≥6.

Statistical analysis. Data were assessed using RevMan (version 5.4;Cochrane) and STATA (version 14.0; StataCorp LP). The HR and 95% CIs were extracted for survival analysis. Odds ratio (OR) and 95% CIs were used as outcome indices for dichotomous variables. Mean differences and 95% Cis were used as outcome indicators for continuous numerical variables. Since all studies included in the present meta-analysis are from completely different groups, the random-effect model was used to analyze data. Cochrane Q test is used to assess whether the observed differences between study results are merely due to chance. A significant P-value (<0.05) indicates heterogeneity. In the event of significant heterogeneity, a subgroup analysis was conducted to investigate the source. Publication bias was evaluated using the Begg's test and funnel plots. P<0.05 was considered to indicate a statistically significant difference.

Results

Characteristics of included studies. Through the literature search, a total of 1,376 relevant studies were obtained. After excluding repeated literature, 976 studies remained. After eligibility evaluation, 10 studies were considered to meet the inclusion criteria of the present meta-analysis (Fig. 1). The characteristics of the included studies are presented in Table I. The 10 included studies were published between 2012 and 2021, of which eight were written in English (2,8,25-30) and 2 in Chinese (31,32). A total of 1,689 patients with CC were included in the present meta-analysis. In all studies, immunohistochemical staining was used to study expression of LC3. NOS was used to assess the quality of the included studies, and all 10 studies were of high quality (Table II).

Meta-analysis of clinicopathological features. The association between LC3 and various clinicopathological traits of CC was evaluated. It was revealed that LC3 expression was associated to histological grade [OR=0.82, 95% CI (0.43, 1.95), P<0.001] and TNM stage [OR=0.91, 95% CI (0.47, 1.77), P<0.001]. However, no association was observed between LC3 expression and sex [OR=1.14, 95% CI (0.90, 1.51); P=0.678], age [OR=0.89, 95% CI (0.67, 1.20), P=0.663], tumor size [OR=0.78, 95% CI (0.30, 2.34), P=0.090], or lymph node metastasis [OR=2.05, 95% CI (1.19, 3.60), 0.250] (Table III).

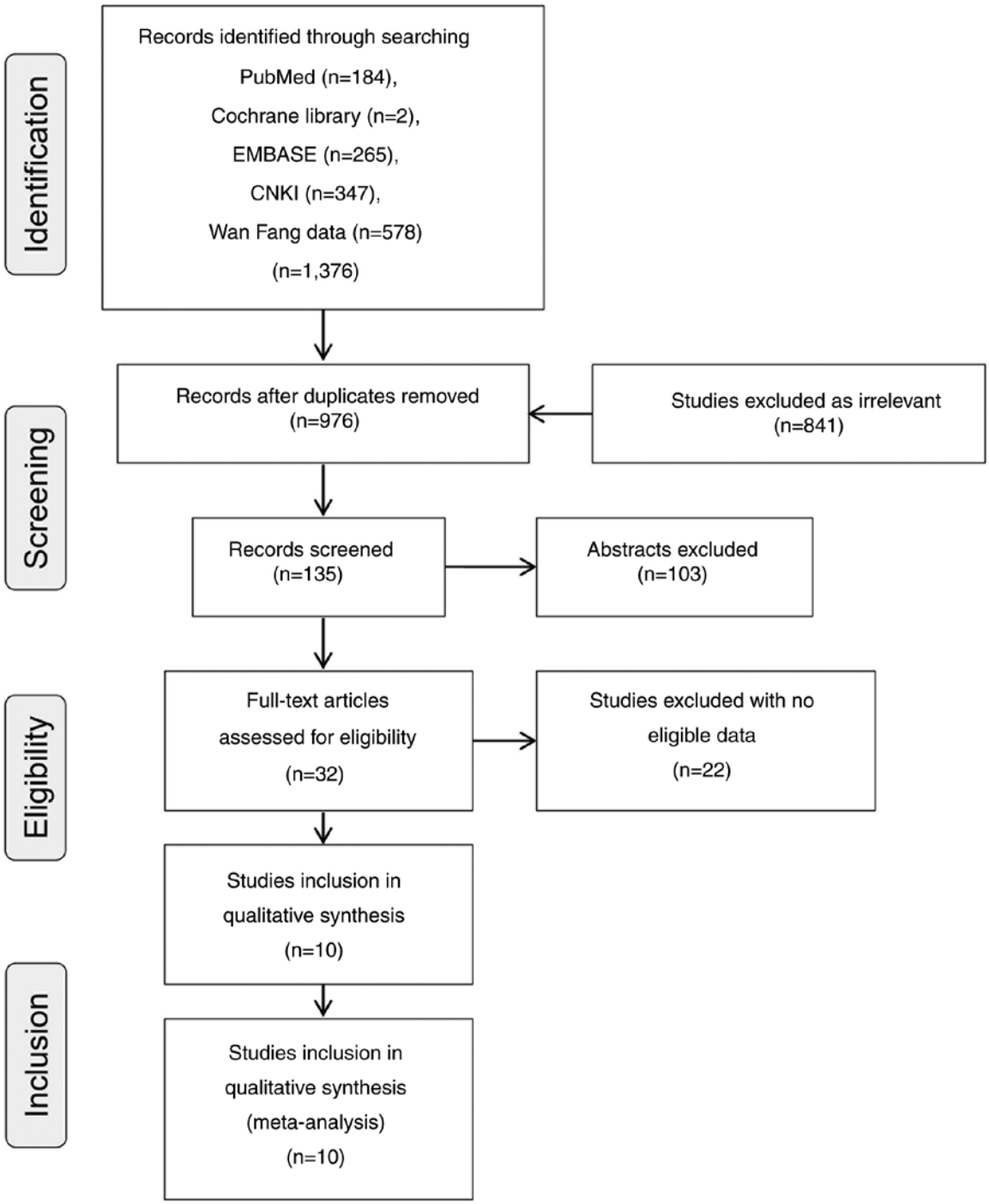

Figure 1. Flow diagram of the literature selection procedure. EMBASE, Excerpta Medica Database; CNKI, China National Knowledge Infrastructure.

Subgroup evaluation. There was heterogeneity in four of the six features studied, including tumor size (I^2=59%, P=0.09), lymph node metastasis (I^2=25%, P=0.25), histological grade (I^2=87%, P<0.001) and TNM stage (I^2=78%, P<0.001; Table III). To investigate the prospective sources of heterogeneity, a subgroup analysis was performed. Because only three studies included tumor size, there was insufficient information for further subgroup analysis. Therefore, according to the sample size and NOS score, only the subgroup analysis of lymph node metastasis, histological grade and TNM stage was performed (Table IV).

Upon classifying the data based on sample size, there was an association between LC3 expression and lymph node metastasis [OR=1.63, 95% CI (1.04-2.56)] as well as TNM stage [OR=0.91, 95% CI (0.47-1.76)] in the subgroup with a small sample size (n≤200), while no significant association was found in the larger sample size subgroup (n>200). Heterogeneity was revealed in the n>200 subgroups for lymph node metastasis, histological grade and TNM stage, with I^2 values of 85.6, 88.7, and 77.5%, respectively.

Based on the NOS score, heterogeneity was revealed in both subgroups of lymph node metastasis (NOS>7, I^2=55.4%; NOS≤7, I^2=65.2%), the low NOS score subgroup of histological grade (NOS≤7, I^2=80.0%) and the low NOS score subgroup of TNM stage (NOS≤7, I^2=87.4%). No heterogeneity

Table I. Newcastle-Ottawa scale for quality assessment.

	Selection				Comparability	Outcome			
First author, year	Exposed cohort	Non-exposed cohort	Ascertainment of exposure	Outcome of interest	Appropriate control used	Assessment of outcome	Follow-up long enough	Adequacy of follow-up	Total score	(Refs.)
Park *et al*, 2013	1	1	1	1	1	1	0	0	6	(25)
Choi *et al*, 2014	1	1	1	1	1	1	1	0	7	(26)
Shim *et al*, 2016	1	1	1	1	1	1	1	1	8	(27)
Schmitz *et al*, 2016	1	1	1	1	2	1	1	0	8	(28)
Wu *et al*, 2015	1	1	1	1	2	1	1	1	9	(8)
Zhao *et al*, 2017	1	1	1	1	1	1	1	0	7	(29)
Guo *et al*, 2019	1	1	1	1	1	1	1	1	8	(30)
Wang *et al*, 2021	1	1	1	1	2	1	1	0	8	(2)
Sui and Feng, 2012	1	1	1	1	2	1	0	0	7	(31)
Li, 2018	1	1	1	1	2	1	0	0	7	(32)

was revealed in the high NOS score subgroups of histological grade (NOS>7, I^2=47.5%) and TNM stage (NOS>7, I^2=0.0%).

Association between LC3 expression and OS in CC. A total of 852 patients from five articles were included to estimate OS value of LC3 expression in CC. No significant heterogeneity (I^2=45% and P=0.12) was revealed (Fig. 2). The results revealed that the overexpression of LC3 was a favorable factor for OS in patients with CC [HR=0.56, 95% CI (0.39, 0.79)].

Publication bias. Begg's test was used to assess the potential publication bias. No publication bias was revealed in sex (P=0.308), age (P=1.000), lymph node metastasis (P=0.060), histological grade (P=0.734) or TNM stage (P=1.000; Fig. 3). In addition, heterogeneity of lymph node metastasis reduced from I^2=53.8% to I^2=31.5% after the study by Wu *et al* (8) was removed.

Discussion

CC remains a medical, social and economic burden in developed countries. Global burden of colorectal cancer in 2020 and 2040: incidence and mortality estimates from GLOBOCAN shows: CC accounting for ~8% of cancer-associated mortalities worldwide (33,34). More than 1.9 million new cases of colorectal cancer and 930,000 deaths are estimated in 2020. CC is a complex malignant tumor involving a variety of cellular signaling pathways, including autophagy cascades. Beclin 1 and LC3 are the most widely studied autophagy-associated proteins in CC (35,36). A previous study revealed that LC3-I and LC3-II were scarce in normal tissues but were strongly positive in ~70% of adenocarcinomas and metastatic tumors (37). Consistent with the results of a previous study (26), the present study revealed that the expression of autophagy-associated proteins maybe a novel prognostic indicator for patients with CC.

The present meta-analysis evaluated the association between the LC3 expression level and the clinicopathological traits and OS in patients with CC. In total, 10 studies with 1,689 patients were included. The findings of the present study indicated that LC3 overexpression was positively associated to lymph node metastasis in CC. Similar to the results of Li *et al* (23), the present study also suggested that LC3 was a protective marker for patients with CC. In total, nine studies were included by Li *et al* (23), and only six of the studies were associated with LC3 expression. Due to the lack of investigation on the clinicopathological characteristics associated to the expression of LC3 in patients with CC, the study by Li *et al* (23) was limited, as the main assessment was the association between LC3 and OS. Therefore, the present meta-analysis investigated the relationship between the expression of LC3 and the diagnosis of CC more comprehensively based on the clinicopathological features of patients with CC (such as tumor size, lymph node metastasis, histological grade and TNM stage).

Furthermore, autophagy may also promote tumor growth under stress conditions, such as hypoxia and starvation (38). Autophagy may have opposing roles in different types of cancer. Previous studies have demonstrated that LC3 expression is associated with developing HCCs (22,39). In the present

Table II. Characteristics of the included studies.

First author, year	Country	Sample size	Sex		Age		Tumor size		Lymph node metastasis		Histological grade		TNM stage		Method	OS data provided	(Refs.)
			Male (+/-)	Female (+/-)	Older (+/-)	Middle aged (+/-)	>5 cm (+/-)	≤5 cm (+/-)	Negative (+/-)	Positive (+/-)	Poorly differentiated (+/-)	Well and moderately differentiated (+/-)	I and II (+/-)	III and IV (+/-)			
Park *et al*, 2013	USA	178	99	79	NA	NA	NA	NA	NA	NA	59	119	NA	NA	IHC	Yes	(25)
Choi *et al*, 2014	South Korea	263	141	122	122	141	NA	NA	99 (68/24)	164 (118/39)	41 (26/14)	222 (160/49)	105 (74/26)	158 (112/37)	IHC	Yes	(26)
Shim *et al*, 2016	South Korea	101	69	32	NA	NA	NA	NA	NA	NA	7 (2/5)	94 (42/49)	52	49	IHC	Yes	(27)
Schmitz *et al*, 2016	Germany	127	66	61	NA	NA	NA	NA	56 (14/42)	63 (20/43)	26 (13/13)	97 (22/75)	15 (3/12)	106 (32/74)	IHC	No	(28)
Wu *et al*, 2015	China	242	127 (113/14)	115 (98/17)	139 (120/19)	103 (91/12)	134 (120/14)	108 (91/17)	133 (107/26)	109 (104/5)	48 (43/5)	194 (168/26)	204 (178/26)	38 (33/5)	IHC	Yes	(8)
Zhao *et al*, 2017	China	526	261 (105/156)	265 (101/164)	269 (106 163)	257 (100/157)	NA	NA	NA	NA	269 (55/214)	257 (151/106)	248 (125/123)	278 (81/197)	IHC	No	(29)
Guo *et al*, 2019	China	68	44 (25/12)	24 (15/4)	13 (6/5)	55 (34/11)	NA	NA	NA	NA	18 (10/5)	44 (30/8)	NA	NA	IHC	Yes	(30)
Wang *et al*, 2021	China	200	110 (97/13)	90 (76/14)	141 (121/20)	59 (52/7)	NA	NA	116 (98/18)	84 (75/9)	79 (69/10)	121 (104/17)	116 (98/18)	84 (75/9)	IHC	No	(2)
Sui and Feng, 2012	China	115	62 (50/12)	53 (45/8)	75 (60/15)	40 (35/5)	101 (81/20)	14 (14/0)	73 (57/16)	42 (38/4)	5 (5/0)	110 (90/20)	NA	NA	IHC	No	(31)
Li, 2018	China	69	42 (30/12)	27 (16/11)	51 (35/16)	18 (11/7)	32 (19/13)	37 (27/10)	49 (28/21)	20 (18/2)	NA	NA	46 (27/19)	23 (19/4)	IHC	No	(32)

The data in the table refers to the number of individuals with the indicated characteristics. NA, not applicable; IHC, immunohistochemistry; (+/-), microtubule-associated protein 1 light chain 3 expression (positive/negative); OS, overall survival.

Table III. Microtubule associated protein 1 light chain 3 clinicopathological descriptions in patients with colorectal cancer.

Clinicopathological characteristic	Number of studies	Number of patients	Pooled OR(95% CI)	Heterogeneity		Model used
				I^2(%)	P-value	
Sex	6	1,208	1.14 (0.90, 1.51)	0.0	0.678	Random
Age	6	1,208	0.89 (0.67, 1.20)	0.0	0.663	Random
Tumor size	3	426	0.78 (0.30, 2.34)	59.0	0.090	Random
Lymph node metastasis	6	994	2.05 (1.19, 3.60)	25.0	0.250	Random
Histological grade	8	1,606	0.82 (0.43, 1.95)	87.0	<0.001	Random
TNM stage	6	1,407	0.91 (0.47, 1.77)	78.0	<0.001	Random

OR, odds ratio; CI, confidence interval.

Table IV. Lymph node metastasis, histological grading and TNM staging subgroup analyses.

A, Lymph node metastasis

Subgroup	Number of studies	Number of patients	Pooled OR (95% CI)	Heterogeneity		Model used
				I^2 (%)	P-value	
Sample, n						
>200	2	491	1.63 (1.04, 2.56)	85.6	0.008	Random
≤200	4	503	2.04 (1.33, 3.26)	19.2	0.287	Random
NOS score						
>7	3	561	2.11 (0.99, 4.52)	55.4	0.106	Random
≤7	3	433	2.22 (0.78, 6.30)	65.2	0.057	Random

B, Histological grade

Subgroup	Number of studies	Number of patients	Pooled OR (95% CI)	Heterogeneity		Model used
				I^2 (%)	P-value	
Sample, n						
>200	3	1,017	0.48 (0.15,1.54)	88.7	<0.001	Random
≤200	5	589	1.37 (0.85, 2.41)	48.6	0.100	Random
NOS score						
>7	5	716	1.30 (0.84, 2.09)	47.5	0.107	Random
≤7	3	890	0.39 (0.13, 1.20)	80.0	0.007	Random

C, TNM stage

Subgroup	Number of studies	Number of patients	Pooled OR (95% CI)	Heterogeneity		Model used
				I^2 (%)	P-value	
Sample, n						
>200	3	1,017	1.43 (0.68, 2.99)	77.5	0.012	Random
≤200	3	390	0.91 (0.47, 1.76)	0.0	0.583	Random
NOS score						
>7	3	563	0.75 (0.42, 1.34)	0.0	0.732	Random
≤7	3	844	1.02 (0.36, 2.85)	87.4	<0.001	Random

OR, odds ratio; CI, confidence interval; NOS, Newcastle-Ottawa scale.

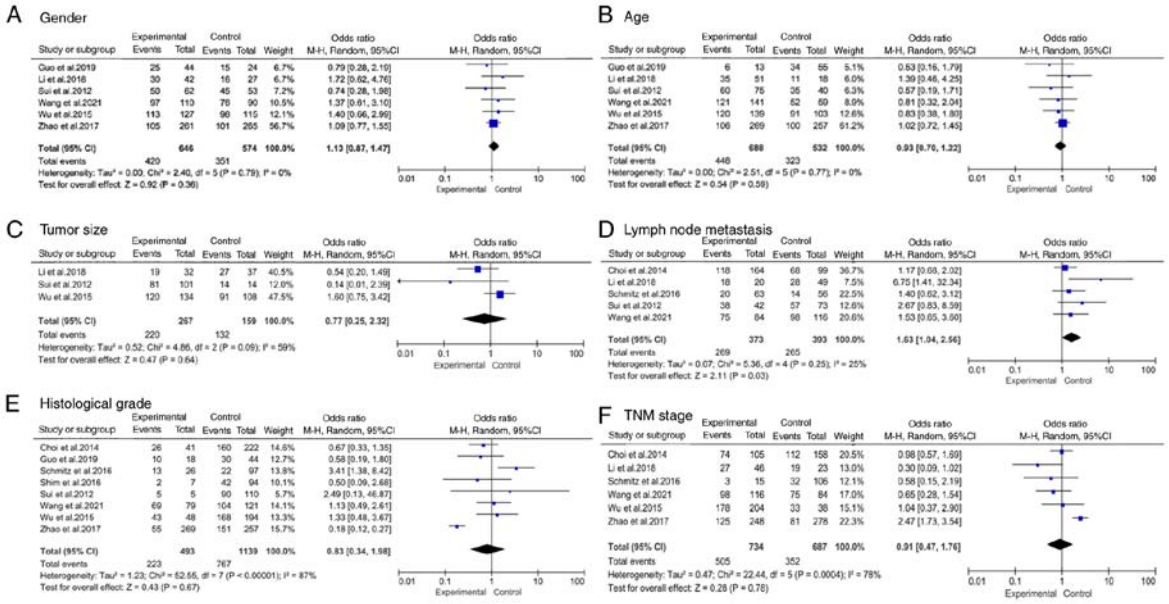

Figure 2. Forest plot of the relationship between microtubule-associated protein 1 light chain 3 expression and overall survival in patients with colorectal cancer. (A) gender, (B) age, (C) tumor size, (D) lymph node metastasis, (E) histological grade and (F) TNM stage. CI, confidence interval. SE, Standard error; df, indicates degree of freedom. IV, Information Value.

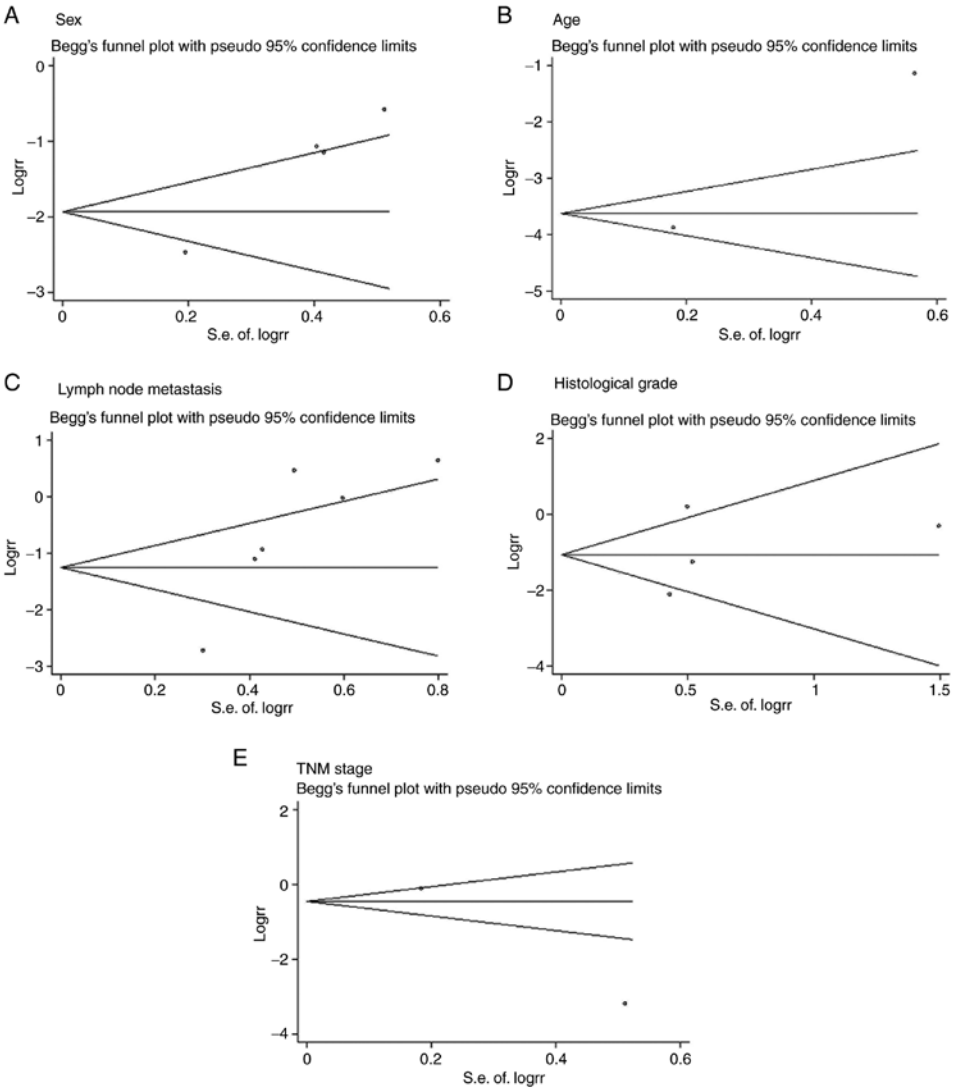

Figure 3. Funnel plot of publication bias in terms of association between LC3 and clinicopathological features of colorectal cancer. (A) Sex, (B) age, (C) lymph node metastasis, (D) histological grade and (E) TNM stage.

study, it was revealed that LC3 may increase the OS of patients with CC.

The present study has reached seemingly contradictory conclusions. It was demonstrated that overexpression of LC3 was associated with lymph node metastasis, which is usually regarded as an unfavorable factor (40). However, in the present study, the high expression levels of LC3 were associated to a favorable OS outcome. Thus, further studies on the role and mechanism of LC3 in CC prognosis need to be conducted. Additionally, the present meta-analysis has several limitations. Firstly, the number of articles and patients included in the present study is small, and further research is required in future. Secondly, the majority of the included studies were conducted in China, which may lead to a potential heterogeneity. Finally, The number of patients included in the studies might have been relatively small, limiting the statistical power and generalizability of the findings, and lymph node metastasis was the only feature associated with LC3 expression in CC.

In summary, the present analysis revealed that LC3 expression was only associated with lymph node metastasis in CC. At the same time, LC3 expression seemed to be a protective indicator for patients with CC. These seemingly contradictory findings need to be verified using a larger sample size with the inclusion of additional high-quality studies.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

NS participated in methodology design, investigation, data curation and writing the original draft. FH and YS contributed to experiment design and writing the original draft. JW contributed to the study design and wrote and edited the manuscript. XC performed the literature review and prepared the figures. LW participated in study conception, supervision, review and editing of the manuscript. NS and LW confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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