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Original Article

Prognostic value of autophagy marker LC3 in esophageal cancer: a meta-analysis

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Abstract: Objective: Autophagy played an important role in the carcinogenesis of esophageal cancer, and LC3 was a popular marker of autophagy. However, the association between LC3 and prognosis of esophageal cancer was controversial. We conducted this meta-analysis to systemically assess the prognostic value of LC3 in esophageal cancer. Materials and methods: Literature searches were performed in Embase and PubMed databases for eligible studies before June 30, 2016. Hazard ratio (HR) was pooled to assess the association of LC3 with overall survival (OS). Odds ratio (OR) was pooled to evaluate the correlation between LC3 and clinicopathological characteristics. Results: A total of six studies involving 775 patients were included for meta-analysis. The pooled result showed that the high LC3 level was significantly correlated with worse OS of esophageal cancer (HR=1.33, 95% CI 1.05-1.68; P=0.018). There was no correlation between LC3 and tumor grade (OR=0.96, 95% CI 0.68-1.36; P=0.822), lymph node involvement (OR=0.99, 95% CI 0.71-1.38; P=0.959) or TNM stage (OR=0.70, 95% CI 0.43-1.13; P=0.142). Conclusion: High LC3 level was correlated with worse prognosis of esophageal cancer, and LC3 might act as a promising autophagy-related prognostic marker of esophageal cancer.

Keywords: Esophageal cancer, autophagy, microtubule-associated protein 1 light chain 3, prognosis, meta-analysis

Introduction

Esophageal cancer is the seventh most prevalent malignant cancer and the sixth chief cause of cancer death worldwide [1]. For the fast progression and late stage in diagnosis, the prognosis of esophageal cancer is dismal. The tumor-node-metastasis (TNM) staging system has made great contributions to the selection of treatment strategies, as well as prediction of prognosis. However, the fact that patients with similar cancer stages tend to have discrepancies in their prognosis indicates that TNM system alone is far from meeting the clinical needs. Some esophageal cancer patients with early stage receiving radical surgery and adjuvant therapy may die of distal metastasis. Thus, it is necessary for us to find new biomarkers to predict the prognosis and provide more information for treatment strategies.

Autophagy, also named as the type II programmed cell death, is an evolutionary conserved process induced by metabolic stress and other stimuli. The most significant character of autophagy is the formation of intracellular double-membrane structure named autophagosome. The autophagosome sequestrates the damaged organelles or stable macromolecules, and fuses with lysosome for turnover of the metabolic product. The basal level of autophagy is essential for the metabolism of cells. However, the role of autophagy in cancer is controversial. On one hand, autophagy acts as an alternative source of energy for tumor cells to cope with nutrition-deficient and oxygen-deficient microenvironment, which promotes the survival of cancer cells [2]. On the other hand, enhanced autophagy causes the arrest of cell cycle by affecting the function of chromosome, interfering with the function of microRNA, and inducing senescence of cancer cells [3, 4].

Microtubule-associated protein 1 light chain 3 (LC3), also known as the mammalian homolog

of yeast Atg8, plays an important role in the formation of autophagosome. LC3 has two subforms, which are cytosolic LC3-I and autophagosome-conjugated LC3-II. As autophagy is activated, the amount of LC3 increases greatly, and LC3-I is transferred into LC3-II to bond with autophagosome. Because of the close association with autophagosome, LC3 is considered to be a potent marker of autophagy. Moreover, the prognostic value of LC3 in various cancers is also investigated. Some researches showed that high LC3 level predicted a better prognosis in cancer patients [5, 6]. However, other researches reported that high LC3 level was correlated with a worse prognosis [7, 8]. Some clinical researches investigated the prognostic role of LC3 in esophageal cancer, but the conclusions were also inconsistent. Here we conducted a meta-analysis to assess the prognostic value of autophagy in patients with esophageal cancer.

Methods

Search strategy

The Embase and PubMed databases were retrieved for studies that focused on the prognostic value of LC3 in esophageal cancer before June 30, 2016. The search strategy included the following terms ("esophageal cancer") and ("LC3" or "microtubule-associated protein 1 light chain 3"). The references of the retrieved studies were also identified. This meta-analysis was registered in the database International prospective register of systematic reviews (PROSPERO) with the register number 42016041932.

Inclusion and exclusion criteria

Studies were primarily included if they satisfied all of the following criteria: (1) Patients were pathologically diagnosed with primary esophageal cancer; (2) The detection marker was LC3; (3) Sufficient information can be extracted to estimate the hazard ratios (HRs) and the 95% confidence intervals (CIs) of overall survival (OS); (4) Manuscript was written in English. Studies were excluded if they satisfied any one of the following criteria: (1) Not written in English; (2) Repeated data; (3) Reviews, letters, animal models, case reports, or laboratory researches; (4) Insufficient information to extract HRs.

Study quality assessment

Three reviewers (Li, Guo and Zhang) independently assessed the quality of included studies according to the Newcastle-Ottawa Quality Assessment Scale [9]. This scale evaluated the included studies by awarding scores, and the total scores were added to assess the quality of included studies.

Data extraction

Two reviewers (Guo and Zhang) evaluated the included studies and extracted data independently. If disagreements arose, a third reviewer (Cui) assessed the studies and collected the data. The best one was adopted after the comparison of three groups of data. The extracted information included the author's name, publication year, country, sample size, tumor stage, detection method, source of HR, cut-off value, and quality score.

Statistical analysis

Patients in the included studies were stratified into high level group and low level group according to the detection level of LC3. Pooled HR and 95% CI of OS were adopted to evaluate the correlation between LC3 and prognosis. HRs were extracted directly from the studies or from the Kaplan-Meier curve according to the method introduced by Pamar [10]. Pooled odds ratio (OR) and 95% CI were adopted to assess the correlation between LC3 and the clinicopathological characteristics, including tumor grade, lymph node involvement, and TNM stage.

The inconsistency index I^2 was adopted to assess the heterogeneity of included studies, $P<0.1$ or $I^2>50\%$ indicated that the heterogeneity was statistically significant [11]. A fixed-effect model was employed if heterogeneity was not significant, whereas a random-effect model was selected if heterogeneity was significant. Egger's bias indicator test and Begg's funnel plot were employed to assess the publication bias [12]. The data was processed with Stata version 12.0 (Stata Corporation, College Station, USA).

Results

Search results and study characteristics

According to the search strategy, 38 studies were identified in PubMed database, and 80

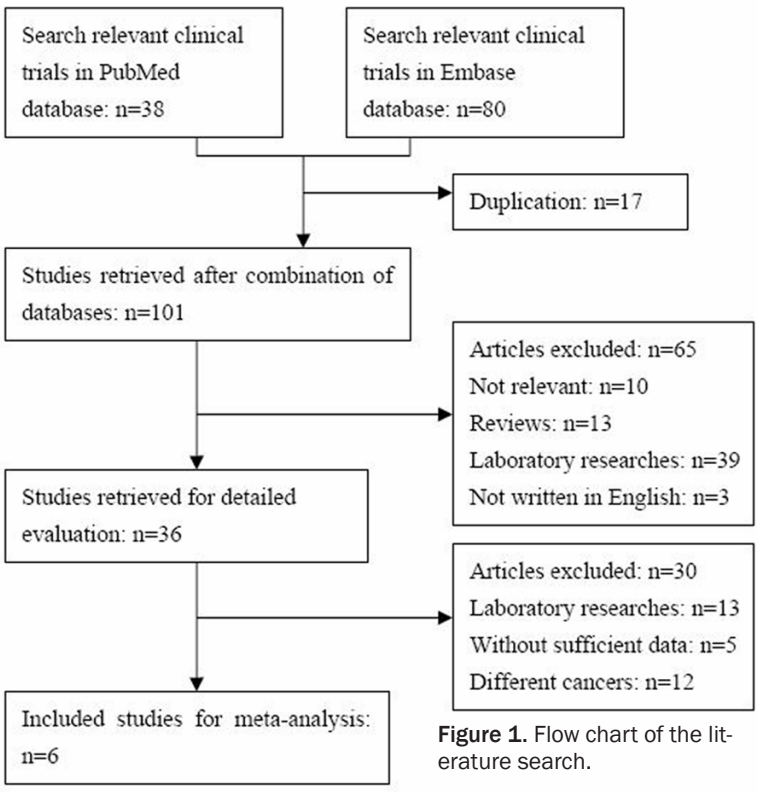

Figure 1. Flow chart of the literature search.

studies were identified in Embase database. By retrieving the titles, 17 studies were removed because of duplication. After reviewing the abstracts, 65 studies were eliminated due to irrelevant content, reviews, laboratory researches, or language limitation. By full-text review, 30 studies were excluded because of irrelevance or incomplete information. Finally, six studies were included for this analysis [13-18] (**Figure 1**).

The six studies included 775 patients (ranging from 43 to 253 for individual study). Four studies were from China, one study was from Switzerland, and one study was from Japan. All the studies detected the expression of LC3 by immunohistochemistry (IHC). Five studies reported the correlation between LC3 and tumor grade, as well as lymph node involvement [13, 15-18]. All the studies reported the association between LC3 and TNM stage. The study quality scores ranged between 6 and 8 (**Table 1**).

LC3 and OS in esophageal cancer

HRs for OS were available in all the studies. The heterogeneity test showed that there was no significant heterogeneity among the six studies (I^2=4.9%, P=0.385), thus a fixed-effect model was applied. The combined analysis indicated that high LC3 level predicted a significantly worse OS in esophageal cancer (HR=1.33, 95% CI 1.05-1.68, P=0.018; **Figure 2**). Because there was no significant heterogeneity, and the conclusion was definitive, the subgroup analyses were not performed.

LC3 and clinicopathological characteristics in esophageal cancer

Then we further investigated the correlation between LC3 and clinicopathological characteristics, including tumor grade, lymph node involvement, and TNM stage. The correlations were not statistically significant between LC3 and tumor grade (OR=0.96, 95% CI 0.68-1.36, P=0.822; fixed-effect model), lymph node involvement (OR=0.99, 95% CI 0.71-1.38; P=0.959; fixed-effect model), or TNM stage (OR=0.70, 95% CI 0.43-1.13, P=0.142, random-effect model; **Table 2**).

Publication bias

We adopted Begg's funnel plot and Egger's bias indicator test to detect publication bias for OS. The publication bias was not observed for OS (Begg's test: p=0.707; Egger's test: p=0.602) (**Figures 3** and **4**).

Discussion

Autophagy plays a crucial in the carcinogenesis and development of esophageal cancer. Recently, more and more researches have focused on the prognostic value of autophagy in cancers, and a serial of autophagy-related markers have been investigated, such as Beclin-1, p62, and ULK1 [19-21]. As one of the most common autophagy-related markers, the prognostic value of LC3 has drawn more attention of researchers. However, the conclusions were controversial. Thus it is necessary to con-

Table 1. Characteristics of included studies

Study	Year	Country	Sample size	Stage	Method	HR	Cut-off	Quality score
Yoshioka [13]	2008	Japan	106	I-IV	IHC	Estimated	IRS	7
Chen [14]	2013	China	150	II-IV	IHC	Estimated	IRS	7
Wang [15]	2013	China	107	II-III	IHC	Reported	50% cells	9
Hao [16]	2014	China	253	I-III	IHC	Estimated	Median	7
Adams [17]	2016	Switzerland	116	I-IV	IHC	Estimated	IRS	9
Chen [18]	2016	China	43	I-IV	IHC	Estimated	30% cells	7

Abbreviation: IHC, Immunohistochemistry; IRS, immunoreactivity score.

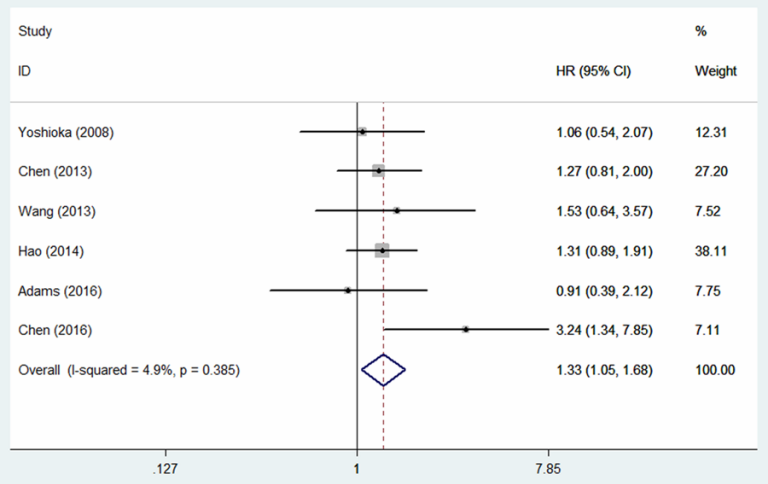

Figure 2. Forest plot for the association between LC3 and OS of patients with esophageal cancer.

firm the prognostic significance of LC3 in esophageal cancer. To the best of our knowledge, this is the first meta-analysis that systemically investigates the prognostic value of LC3 in esophageal cancer. In this meta-analysis, six studies with 775 patients were included for meta-analysis. A pooled HR 1.33 with 95% CI of 1.05-1.68 indicated that high LC3 level was significantly correlated with worse overall survival of esophageal cancer. Because there was no significant heterogeneity, subgroup analyses were not conducted. Moreover, LC3 was not associated with the tumor grade, lymph node involvement or TNM stage of esophageal cancer. The conclusion indicated that LC3 might be a promising prognostic marker of esophageal cancer.

The LC3 level is specifically correlated with the functional basal autophagy in cancer cells [22]. As autophagy is up-regulated, the synthesis of LC3 increases, and LC3-I is converted into LC3-II to participate in the formation of autophagosome. In addition to LC3, a series of other autophagy-related markers are involved in the formation and regulation of autophagy, such as Beclin-1, ULK1, and p62. However, the prognostic roles of these markers in esophageal cancer are inconsistent. This may be because they not only participate in autophagy, but also play important roles in other regulatory pathways. For example, the Beclin-1 not only participates in autophagy, but also has the ability to interact with Bcl-2 and promote apoptosis by inhibiting the function of Bcl-2 [23]. The p62 induces apoptosis by promoting the accumulation of Caspase-8 [24]. Three researches investigated the prognostic role of Beclin-1 in esophageal cancer [14, 18, 25], and two researches investigated the prognostic role of ULK1 in esophageal cancer [26, 27], but the conclusions were divergent. A meta-analysis showed that elevated Beclin-1 predicted a worse prognosis in colorectal cancer, but the prognostic role of Beclin-1 in esophageal cancer is inconsistent [28]. Although several autophagy-related markers are involved in the autophagy of esophageal cancer, only LC3 shows the prognostic correlation with esophageal cancer.

Moreover, we found that LC3 was not associated with the tumor grade, lymph node involvement, or tumor stage of esophageal cancer. Some researches showed that LC3 was up-regulated in cancer tissue compared with normal tissue, which indicated that autophagy and LC3 might participate in the carcinogenesis [29]. However, there were divergences between LC3

Table 2. Correlation analyses on high LC3 level and clinicopathological characteristics of esophageal cancer

Classification	No. of cohorts	No. of patients	OR (95% CI)	*P* value	Heterogeneity	
					I^2 (%)	*P* value
Tumor grade (T3-4 vs T1-2)	5	625	0.96 (0.68, 1.36)	0.822	11.3%	0.342
Lymph node (N1 vs N0)	5	625	0.99 (0.71, 1.38)	0.959	36.9%	0.175
TNM stage (III/IV vs I/II)	6	775	0.70 (0.43, 1.13)	0.142	56.8%	0.041

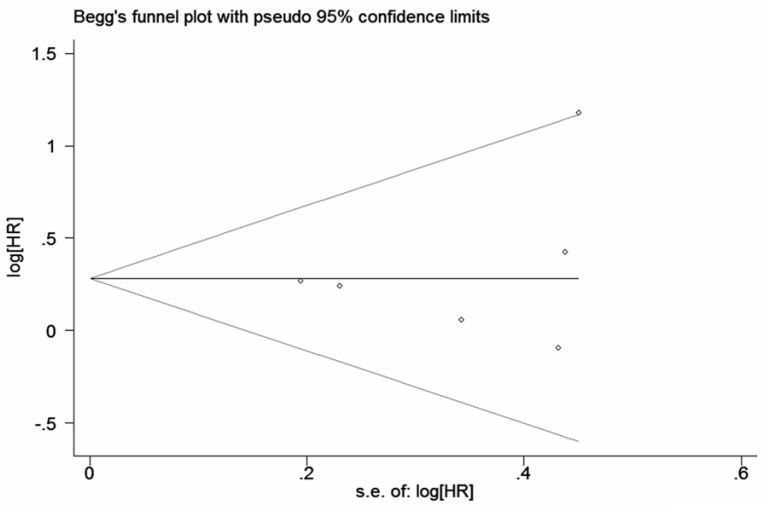

Figure 3. Publication bias of included studies for Begg's funnel plot of OS.

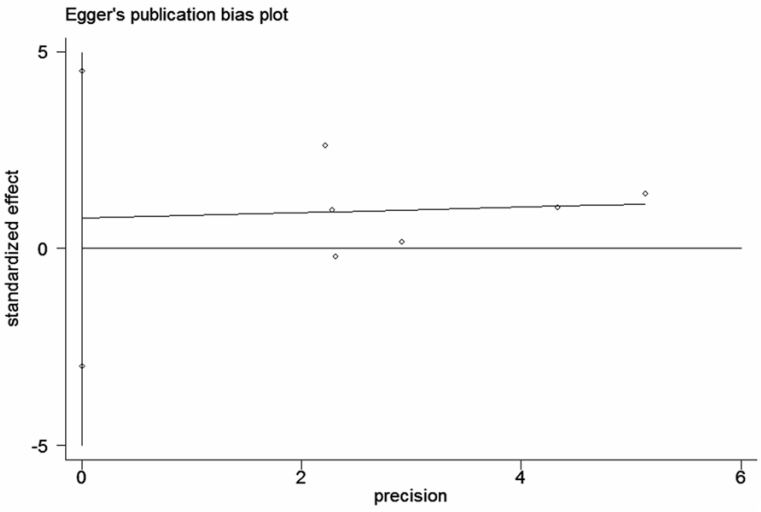

Figure 4. Publication bias of included studies for Egger's plot of OS.

and clinicopathological characteristics of cancers. Some researches showed that LC3 was correlated with advanced stage of cancers, while other researches showed that LC3 was not associated with clinicopathological characteristics of cancers [30, 31]. Our analysis revealed that LC3 was not correlated with the tumor grade, lymph node involvement, or tumor stage of esophageal cancer. This indicated that LC3 might play different roles during the carcinogenesis stage and progression stage of esophageal cancer. However, more researches are required to further investigate the detailed mechanism.

There were some limitations that should not be ignored. First, the eligible studies were limited. Only six studies were included, and the sample sizes were not large enough. Second, most HR data were estimated from Kaplan-Meier curves, and the study would be better if more HR data were reported explicitly. Third, most studies were from China, and the conclusion would be more reliable if some multi-centre studies were performed. Moreover, there was no unique cut-off value to distinguish between high level and low level of LC3 expression, which made it difficult to use in clinical practice. It is crucial to establish definitive criteria to classify high LC3 level patients and low LC3 level patients.

In conclusion, our meta-analysis showed that high LC3 level predicted a significant worse OS in esophageal cancer. Moreover, LC3 level was not associated with tumor grade, lymph node involvement, or tumor stage of esophageal cancer. The conclusion indicated that LC3 might

act as a promising autophagy-related prognostic marker of esophageal cancer.

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Disclosure of conflict of interest

None.

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