RESEARCH ARTICLE

Prognostic Significance of Beclin-1 Expression in Colorectal Cancer: a Meta-analysis

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Abstract

Objective: Beclin-1 has recently been observed as an essential marker of autophagy in several cancers. However, the prognostic role of Beclin-1 in colorectal neoplasia remains controversial. Our study aimed to evaluate the potential association between Beclin-1 expression and the outcome of colorectal cancer patients. Materials and Methods: All related studies were systematically searched in Pubmed, Embase, Springer and Chinese National Knowledge Infrastructure databases (CNKI), and then a meta-analysis was performed to determine the association of Beclin-1 expression with clinical outcomes. Finally, a total of 6 articles were included in our analysis. Results: Our data showed that high Beclin-1 expression in patients with CRC was associated with poor prognosis in terms of tumor distant metastasis (OR=2.090, 95% CI=1.061-4.119, *p*=0.033) and overall survival (RR=1.422, 95% CI=1.032-1.959, *p*=0.031). However, we did not found any correlation between Beclin-1 over-expression and tumor differentiation (OR=1.711, 95% CI=0.920-3.183, *p*=0.090). In addition, there was no evidence of publication bias as suggested by Egger's tests for tumor distant metastasis (*p*=1.000), differentiation (*p*=1.000) and OS (*p*=0.308). Conclusions: Our present meta-analysis indicated that elevated Beclin-1 expression iss associated with tumor metastasis and a poor prognosis in patients with CRC. Beclin-1 might serve as an efficient prognostic indicator in CRC, and could be a new molecular target in CRC therapy.

Keywords: Beclin-1 - colorectal cancer - prognosis - meta-analysis

Asian Pac J Cancer Prev, **15 (11)**, 4583-4587

Introduction

Colorectal cancer is the second leading cause of male cancer-related death and the third leading cause for female cancer-related death worldwide. Every year, over 1.2 million new cancer cases occurred, leading to more than 600 thousand deaths (Jemal et al., 2011). Although operation/chemotherapy and radiotherapy have made a great progress recently, the clinical outcome of CRC is still poor (Kekelidze et al., 2013; Zafar et al., 2013; Tong et al., 2014). Therefore, developing novel and effective therapeutic methods is essential to reduce CRC mortality (Khiewkhern et al., 2013).

Autophagy is a homeostatic process that enables the recycling of long-lived proteins or damaged organelles, which is induced in tumor cells to maintain survival in a setting of stress due to increased metabolic demands, a hypoxic microenvironment or cytotoxic agents (Klionsky and Emr, 2000; Mizushima et al., 2008). The autophagic cancer cell response to ionizing radiation and chemotherapy seems to affect the efficacy of chemotherapy and radiotherapy (Zois and Koukourakis, 2009). Studies have shown that inhibition of autophagy in tumor cells can enhance chemotherapy-induced cell death (Chen et al., 2013; Zhang et al., 2013). Though oncogenes and tumor suppressor genes are also involved in the regulation of autophagy, the role of autophagy in the metastasis and prognosis of human colorectal cancer are still poorly understood (Maiuri et al., 2009).

Beclin-1, the mammalian orthologue of the yeast Apg6/Vps30 gene, is considered as the first identified autophagy gene product (Zeng et al., 2006; Cao and Klionsky, 2007). It is involved in nucleation and locus on chromosome 17q21, which is isolated as a Bcl-2-interacting protein (Miracco et al., 2007). Recently, Beclin-1 has been shown to be over-expressed in many types of cancer, including gastric cancer (Geng et al., 2012), pancreatic adenocarcinoma (Kim et al., 2011) and hepatocellular cell carcinoma (Song et al., 2004). Over-expression of Beclin-1 enhanced tumor aggressive clinical behavior in colorectal cancer. Guo et al reported that down-regulation of Beclin-1 contributed to a longer median progression free survival (Guo et al., 2011). However, the function of Beclin-1 in colorectal cancer was still controversial.

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For example, Koneri et al transfected Beclin-1 gene into HT29 colon cancer cells, and demonstrated that the percentage of G0/G1-pahse cells was significantly higher than that in mock transfected cells (Koneri et al., 2003; Koneri et al., 2007). Similar studies also demonstrated that over-expression of Beclin-1 inhabited colorectal cancer cell growth (Chen et al., 2013), and the higher level of Beclin-1 was strongly associated with longer survival(Li et al., 2009). So no certainty outcomes were determined up to now.

In order to determine the potential function of Beclin-1 and address controversial issues in CRC, our present meta-analysis was performed.

Materials and Methods

Literature search

Up to December 2013, a literature search of Pubmed, Embase, Springer and CNKI was performed to identify articles using the following search terms and their combinations: "Beclin-1" or "ATG6", "colorectal cancer" or "colon cancer", and "over survival". Studies that were included had to meet the following criteria: (1) articles were written in English or Chinese; (2) studies were published as original research; (3) there was quantitative information reporting the relationship between Beclin-1 expression and either prognostic factors or OS; (4) studies must be the full-text manuscripts.

Accordingly, The excluding criterion were as follows: (1) review articles, simple commentaries, case reports, or unpublished reports, (2) not offering the sources of case and controls, (3) fewer than 50 patients or follow-up less than 1 year. (4) researched by RT-PCR and not acquired in full text.

Data extraction

In order to avoid bias in the data-abstraction, 2 investigators independently abstracted the data (Ye Han and Xiaofeng Xue), and differences in the extraction of data were checked by the third investigator (Qiaoming Zhi). The information was extracted from the eligible articles, including containing author, the country of author, the year of publication, tumor stage, number of patients, experimental method used, immunopositivity of beclin-1 and tumor location. Based on the objective, the association between Beclin-1 expression and degree of differentiation was clarified, as well metastasis of tumor. And the relationship between Beclin-1 expression and OS was investigated to estimate the RR of patients' 20 months-survive.

Statistical analysis

OR with 95%CI was used to estimate the association between Beclin-1 expression and tumor metastasis, as well degree of differentiation. To facilitate analysis, we combined the following dates into the single categories: Beclin-1-negative and Low; meditate and poor differentiation. RR and 95%CI were used to measure the impact of Beclin-1 expression on survival of colorectal cancer patients. When extracting the survival data, because of some studies not given these data clearly, we calculated from available data or Kaplan-Meier survive cure as described by Parmar(Parmar et al., 1998).

Heterogeneity was assessed by the Chi-squared test and *p* value in our meta-analysis. Using I^2 value to evaluate the heterogeneity, fixed-effect model was used if there was I^2 =0-50%, which means no significant heterogeneity. Otherwise, the random-effects model was applied. Funnel plots and Egger' linear regression test were used to assess evidence for publication bias. All *p* values were two-side, being statistically significant when *p* value less than 0.05. All the statistical analyses were performed by STATA Version 11.0 (Stata Corporation, College Station, TX, USA)

Results

Study characteristics

Titles and abstracts of 145 studies were carefully reviewed to exclude those that were clearly irrelevant with the association of beclin-1 expression and CRC. As shown in Figure 1, a total of 26 articles were initially included by the literature searching strategy above. 7 potentially candidate studies were fully reviewed with the full text. Among them, 1 study was excluded because of the duplicated data about Beclin-1 expression. Finally, 6 articles were eligible for our present meta-analysis (Ahn

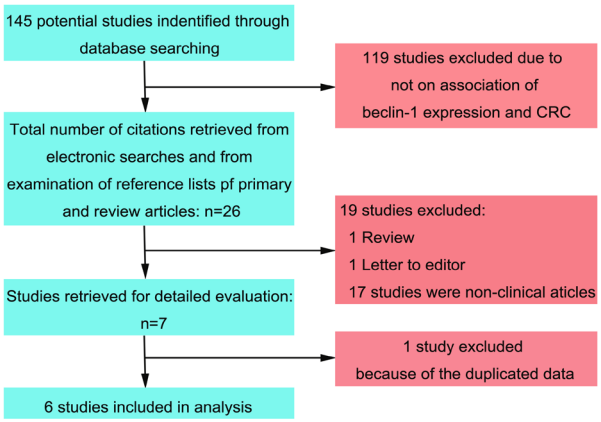

Figure 1. Flow Diagram of Identifying Potential Studies in our Analysis

Table 1. Characteristics of The Included Studies

Study	Country	Year	Tumor stage	Number of patients	Method	Location
Ahn et al.	Korea(S)	2007	I-IV	103	IHC	Colon or Rectum
Li et al.	China	2008	III-IV	115	IHC	Colon or Rectum
Koukourakis et al.	UK	2010	II-III	155	IHC	Colon or Rectum
Guo et al.	China	2011	II-IV	85	IHC	Colon or Rectum
Jae et al.	USA	2012	II-III	178	IHC	Colon
Sui et al.	China	2012	I-IV	115	IHC	Colon or Rectum

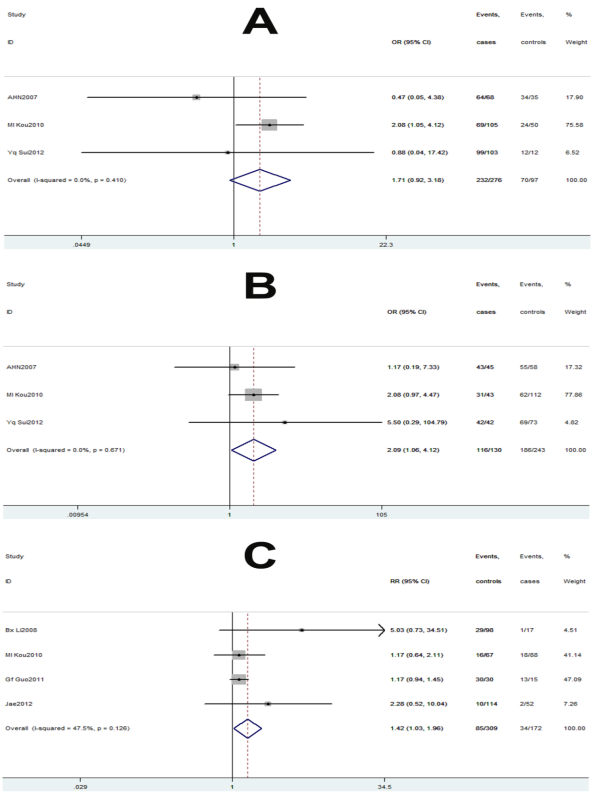

Figure 2. Forrest Plot of ORs for the Association of Lgr5 Expression with (A) Tumor Differentiation; (B) Tumor Distant Metastasis; (C) Overall Survival of Patients

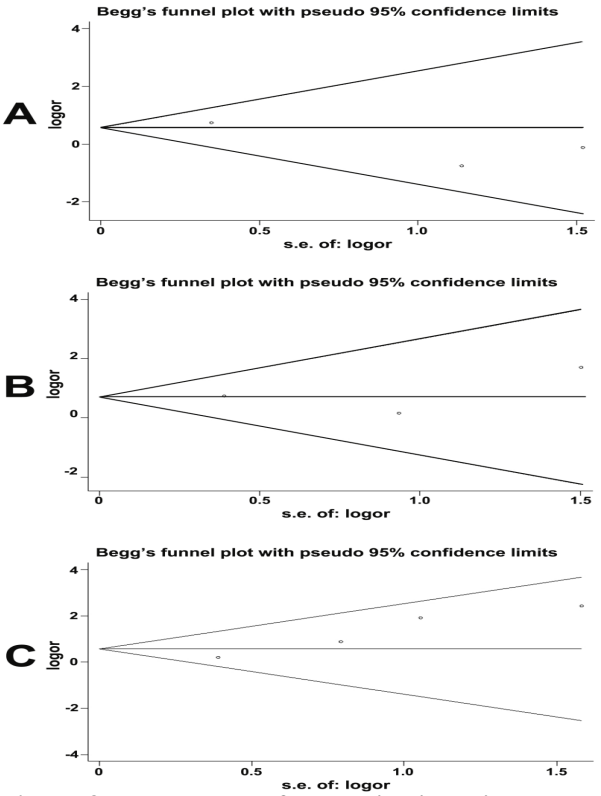

Figure 3. Funnel Plots for Publication Bias. All the graphical funnel plots appeared to be symmetrical. **A)** Tumor differentiation; **B)** Tumor distant metastasis; **C)** Overall survival of patients

Table 2. Results of Meta-Regression Analysis Exploring Source of Heterogeneity with Overall Survival

Covariates	Univariate analysis		
	Co-efficient	SE	*P* value
Chemotherapy	0.6	1.15	0.65
Cutoff of Beclin-1 positive	-0.064	0.56	0.91
Tumor location	-0.85	0.90	0.44

*The dependent variable is the RR for overall survival (OS) from each study. Weights have been assigned according to the estimated variance of RR. SE, standard error of the coefficient

et al., 2007; Guo et al., 2011; Koukourakis et al., 2010; Li et al., 2009; Myung Park et al., 2013; Sui and Feng, 2012).

Immunohistochemistry (IHC) was the only method to evaluate Beclin-1 expression in CRC specimens. The main characteristics of these 6 studies were described in Table 1. The reported follow-up period of patients ranged from 3 to 96 months. Clinicopathological factors were extracted from the main 3 papers to evaluate the tumor distant metastasis and degree of differentiation. 4 publications dealt with the association between the Beclin-1 expression and OS.

Correlation of beclin-1 expression with clinicopathological parameters

As shown in Figure 2A, 3 eligible studies showed that the Beclin-1 expression was not associated with the differentiation of tumor (OR=1.711, 95%CI=0.920-3.183, p=0.090, fixed-effect), with no significant heterogeneity. Our analysis indicated that high Beclin-1 expression in patients with CRC was associated with poor prognosis in terms of tumor distant metastasis (OR=2.090, 95%CI=1.061-4.119, p=0.033, fixed-effect) (Figure 2B).

Correlation of beclin-1 expression with overall survival

In 4 included studies, due to RRs not given directly, the data and figures were extracted from original papers. Because of the heterogeneity being no significant (p>0.05), the fixed-effect model was adopted to calculate the RRs. Based on the meta-analysis, high Beclin-1 level was statistically related to the overall survival (RR=1.422, 95%CI=1.032-1.959, p= 0.031) (Figure 2C). The explanatory variables did not significantly influence RR estimates for OS (Table 2).

Publication bias

No significant publication bias was confirmed to exist in tumor metastasis and differentiation, because both of their P value was larger than 0.05 in Egger's test (Figure 3A and B). There was also no evidence for obvious publication bias in OS (Egger's test, p= 0.308) (Figure 3C). The finding was another strong evidence to verify that Beclin-1 was an independent prognostic factor for CRC patients.

Discussion

Beclin-1 is the first identified autophagy-related gene that could reflect the level of autophagy(Cao and Klionsky, 2007; Zeng et al., 2006). It plays a crucial role in the process of tumorigenesis. Experiments in vitro had reported that

Beclin-1 could be as a reliable marker in monitoring the prognosis in CRC. However, conflicting results have been also reported from different laboratories(Guo et al., 2011; Li et al., 2009). In our meta-analysis, higher Beclin-1 expression was positively linked to tumor distant metastasis, which was also confirmed by Ahn's study(Ahn et al., 2007). In Geng's study, Beclin-1 expression was also significantly correlated with depth of invasion and lymph node metastasis in other gastrointestinal cancers(Geng et al., 2012). All these results indicated that Beclin-1 might be involved in the metastasis of colorectal cancer. In term of differentiation, it was no significant association with Beclin-1 expression in CRC. Whereas, a novel meta-analysis showed that Beclin-1 expression was different in intestinal- and diffuse-type gastric cancer(Xia et al., 2013). We conjectured that Beclin-1 might play different unknown roles in the tumor differentiation for different cancer types. Moreover, our date showed that high Beclin-1 level was also statistically associated with the overall survival in colorectal cancer. Exploring sources of heterogeneity of OS (Table 2), we found that Beclin-1 over-expression was associated with reduced survival in the CRC patients with chemotherapy, while among the patients without chemotherapy, high Beclin-1 levels led to longer overall survival. The result might indicate that selective autophagy might help the tumor cell get rid of the invading chemotherapy drugs, and activating autophagy could be a new therapeutic method.

In interpreting the results from our meta-analysis, there were some limitations should be addressed. Firstly, other significant clinicopathologic parameters, including the tumor size, TNM stage and type of tumor, were not discussed in our analysis because of inadequate data. But we believe that there will be more clinical researches in the future that can effectively focus on these parameters. Secondly, IHC was the only applied method, but the cutoff value was defined differently (Table 1). Thirdly, the data of OS was extracted from survival curves rather than original data of variance. These factors could limit our estimation of potential interactions. Finally, with more studies focused on autophagy, more reliable prognostic biomarkers, combined with Beclin-1, will be applied for predicting the clinical outcome of CRC. And co-expression of various biomarkers associated with patient survival may be more meaningful for clinical application in CRC.

To our knowledge, this meta-analysis is the first one to evaluate the prognostic role of Beclin-1 expression in colorectal cancer. Though larger well-designed studies with more ethnic groups and larger population studies are required, our present meta-analysis indicated that elevated Beclin-1 over-expression was associated with tumor metastasis and poor prognosis in patients with CRC. Beclin-1 might serve as an efficient marker for prognostic indicator, and could be a new molecular target in CRC therapy.

Acknowledgements

This study was supported by a grant from the National youthful Science Foundation of China (No. 81201905 and 81302147), the National Science Foundation of Jiangsu Province, China (No. BK20130270).

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