Original Article
High level of microtubule-associated protein light chain 3 predicts poor prognosis in resectable esophageal squamous cell carcinoma

Chong-Li Hao[1,5*], Yong Li[3,4*], Hao-Xian Yang[2,3], Rong-Zhen Luo[4], Ying Zhang[3], Mei-Fang Zhang[4], Yu-Feng Cheng[1], Xin Wang[2]

[1]Oncology Center, Qilu Hospital of Shandong University, Jinan City, Shandong Province, China; [2]Department of Thoracic Surgery, Sun Yat-Sen University Cancer Center, Guangzhou City, Guangdong Province, China; [3]State Key Laboratory of Oncology in South China, Guangzhou City, Guangdong Province, China; [4]Department of Pathology, Sun Yat-Sen University Cancer Center, Guangzhou City, Guangdong Province, China; [5]Oncology Center, Tengzhou Central People's Hospital, Tengzhou City, Shandong Province, China. *Equal contributors.

Abstract: Microtubule-associated protein light chain 3 (LC3) is a key mediator bridging autophagy, apoptosis and differentiation. However, its role and clinical significance in resectable esophageal squamous cell carcinoma (ESCC) is still scanty. The purpose of this study was to investigate the clinical significance of LC3 by immunohistochemistry in a group of patients with ESCC treated with surgical resection. Tissue microarray that included 253 surgically resected ESCC specimens was successfully generated for immunohistochemical evaluation. The clinical/prognostic significance of LC3 expression was analyzed statistically. The association of LC3 expression with the ESCC survival rate was assessed by Kaplan-Meier and Cox proportional-hazards regression. The results showed that the immunostaining of LC3 was distributed in cytoplasm and plasma-membrane. Significantly high LC3 expression was found in ESCC cells compared with that of normal esophageal epithelial cells. Patients with low expression of LC3 demonstrated higher overall survival compared with those with high expression of LC3 (mean of 71.1 months versus 55.5 months, $P = 0.022$). A similar result was observed for disease-free survival (mean of 68.7 months versus 51.8 months, $P = 0.021$). In subgroup analysis, LC3 expression could stratify pN0 patients with ESCC. Multivariate analysis showed that the level of LC3 expression was an independent prognostic factor in ESCC (RR = 1.407, $P = 0.049$). This paper shows high level of LC3 suggests poor prognosis for resectable ESCC patients.

Keywords: Esophageal cancer, immunohistochemistry, LC3, surgery

Introduction

Esophageal squamous cell carcinoma (ESCC) is one of the most aggressive malignancies of the digestive tract [1, 2]. Surgical resection is still as the mainstay strategy employed for operable ESCC. Despite the great advances has been achieved in multimodal therapy, its five-year survival rate remains unsatisfactory [3-5]. Discovering suitable biomarkers will probably be a key to monitoring cancer recurrence or screening high risk population of ESCC, giving information on the need for adjuvant or neoadjuvant therapy.

LC3, microtubule-associated protein light chain 3, was originally identified as a protein that co-purifies with large microtubule associated with MAP1A and MAP1B from rat brain [6]. LC3 is an autophagosomal orthologue of yeast Atg8, with approximately 30% amino acid homology with Atg8 [7, 8]. It exists in two forms, LC3-I and LC3-II (molecular weight, 18 and 16kD, respectively), localized in the cytoplasm. LC3 is now widely used as a specific molecular marker to monitor autophagosome formation. Up-regulation of LC3 expression was observed in the presence of various stresses such as genomic injury, hypoxia, viral/bacterial infection, starvation [9].

Currently, numerous studies have been investigated on the role of autophagy in cancer development and cancer treatment. Accumulating data provide evidence that autophagy is

involved in tumor suppressor pathways. Beclin-1, an essential mediator of autophagy, was confirmed as a tumor suppressor in heterozygous mouse models [10, 11], and intensive expression of Beclin-1 was found in breast, colorectal, and gastric cancers [12-14]. Furthermore, higher expression of Beclin-1 has shown favorable survival than the lower ones in ESCC patients [15]. However, other studies supported the idea that autophagy enhances tumor progression and protects cancer cells from anticancer therapies. As tumors grow, autophagy may contribute to cancer cells survival under nutrient deprivation and hypoxia conditions [16-19]. Knockdown of autophagy, in combination with tamoxifen or 4-hydroxy-tamoxifen (4-OH-T), resulted in decreased cell viability of estrogen receptor-positive breast cancer cells [20, 21]; Inhibition of autophagy along with irradiation lead to enhanced cytotoxicity of radiotherapy in resistant cancer cells [22]. Thus, the role of autophagy in tumor suppression and development is still controversial.

LC3, a specific marker of autophagy, has been examined in gastrointestinal cancers [23], but the evidence related to survival is still scanty in ESCC. Therefore, we performed this study to evaluate and explore the possible relation between the LC3 expression and prognosis in a large cohort of ESCC (253 cases) by immunohistochemistry.

Materials and methods

Patient selection

This study was approved by the medical ethics committee of Sun Yat-Sen University Cancer Center. Two hundred and sixty-five primary ESCC patients who underwent surgery at the Department of Thoracic surgery, Cancer Center, Sun Yat-Sen University, between October 2000 and April 2007 were eligible for enrollment in the study. The histologic grade and clinical stage of the tumors were defined according to the 7th edition of the TNM classification of the International Union Against Cancer [24]. The cases selected in this study fulfilled the following criteria: (a) newly diagnosed cancer of the esophagus without previous treatment; (b) histologically confirmed primary thoracic ESCC; (c) no distant metastases, including supraclavicular or celiac lymph nodes metastases; (d) underwent a complete surgical resection (R0)

at our cancer center; (e) adequate clinical information and follow-up data were available. Patients with a non-curative resection (R1) or died from postoperative complications were excluded from the study. Patients with neoadjuvant or adjuvant therapy were also excluded. In our cancer center, patients with ESCC that has invaded the airway or major vessels (such as the thoracic aorta) or accompanied by visceral metastasis are not indicated for surgery. The tumor specimens and paracancerous samples were obtained as paraffin blocks from the Bank of Tumor Source at our cancer center. Clinical data were obtained from hospital records after surgery. All the patients were followed up in May 2010 to determine their current status.

Tissue microarray construction

Tumor tissue samples from 265 ESCC cases were collected, fixed in formalin, and embedded in paraffin. H&E-stained sections from a single random block from each patient were reviewed by a senior pathologist (R-Z Luo) to define representative tumor regions. Two targeted core samples of each specimen were obtained using a tissue array instrument (ALPHELYS Minicore instruments, France). Briefly, tissue cylinders with a diameter of 10 mm were punched and arrayed on a recipient paraffin block. Sections (5 μm) of the tissue array (recipient) block were cut and placed on class slides. After the exclusion of cores with inadequate tissue following sectioning and tissue transfer, the final immunohistochemical analyses included cores from 253 ESCC cases. Each of the 253 different ESCC cases contributed to the biomarker analyses. Among the 253 cases, formalin-fixed paracancerous normal esophageal tissues were available for 56 cases, which served as controls. The microarray for the normal esophageal tissues was constructed according to the same method described above.

Immunohistochemistry (IHC) staining and assessment

IHC staining was performed using TMA sections that were rehydrated via a graded alcohol series. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide for 15 minutes. For antigen retrieval, the TMA slides were boiled in tris(hydroxymethyl) aminomethane-EDTA buffer (pH 8.0) in a pressure cooker
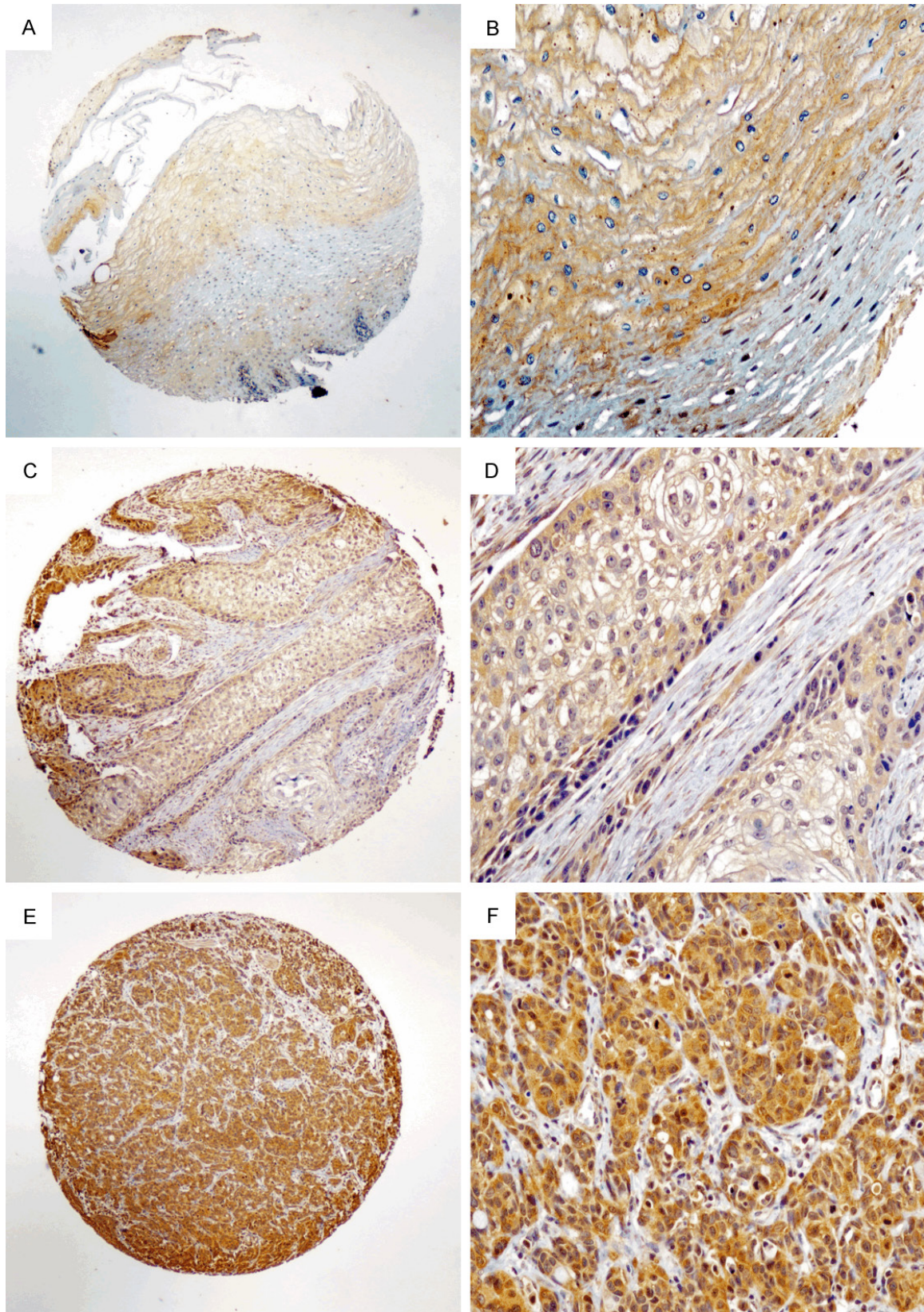
Figure 1. LC3 expression by immunohistochemical staining. (A, B) Normal esophageal mucosa demonstrated low expression of LC3 protein in the cytoplasm of all esophageal squamous cells (magnification: A, ×40, B, ×200). (C, D) An ESCC case demonstrating a low expression level of p300 (magnification: C, ×40, D, ×200). (E, F) High expression level of LC3 detected in ESCC (magnification: E, ×40, F, ×200).

Table 1. Relationship between LC3 expression and clinicopathological variables

Variables	Case number	LC3 Expression (%)		
		Low	High	P-value[d]
Age (years)				
≤60	153	79 (51.6)	74 (48.4)	0.955
>60	100	52 (52.0)	48 (48.0)	
Gender				
Male	183	97 (53.0)	86 (47.0)	0.528
Female	70	34 (48.6)	36 (51.4)	
Surgery				
Standard	155	82 (52.9)	73 (47.1)	0.653
Three-incision	98	49 (50.0)	49 (50.0)	
Tumor location				
Upper	13	6 (46.2)	7 (53.8)	0.902
Middle	174	90 (51.7)	84 (48.3)	
Lower	66	35 (53.0)	31 (47.0)	
Histologic grade				
G1	58	32 (55.2)	26 (44.8)	0.155
G2	163	78 (47.9)	85 (52.1)	
G3	32	21 (65.6)	11 (34.4)	
pT category				
1	5	1 (20.0)	4 (80.0)	0.238
2	60	35 (58.3)	25 (41.7)	
3	185	93 (33.1)	92 (66.9)	
4	3	1 (33.3)	2 (67.7)	
pN category				
0	135	70 (51.9)	65 (48.1)	0.980
1/2/3	118	61 (51.7)	57(48.3)	
pTNM stage				
I	9	7 (77.8)	2 (22.2)	0.195
II	145	77 (53.1)	68 (46.9)	
III	99	47 (47.5)	52 (52.5)	

[d]Pearson's χ^2 test; standard: left thoracotomy, three-incision: thoracic-abdominal-cervical anastomosis; Fisher's χ^2 test.

for 20 min. Nonspecific binding was blocked with 10% normal goat serum for 20 min. The TMA slides were incubated with rabbit anti-LC3 antibody (NB100-2220, 1:400 dilution, Novus) for 12 hours at 4°C in a moist chamber. Subsequently, the slides were sequentially incubated with biotinylated rabbit anti-mouse immunoglobulin antibody at a concentration of 1:100 for 30 min at 37°C and then with a streptavidin-peroxidase conjugate for 30 min at 37°C and 3'-3'diaminobenzidine as the chromogen substrate. The nucleus was counterstained using Meyer's hematoxylin. The negative control was obtained by replacing the primary antibody with normal rabbit IgG. Positive

expression of LC3 in ESCC and normal esophageal mucosa cells exhibited a primarily cytoplasm pattern (Figure 1). Internal positive and negative controls, including normal squamous mucosa of the esophagus from non-cancer patients, were utilized as available to further support the staining patterns. Two independent observers (R-Z Luo and M Li) blinded to the clinicopathological information performed the immunoreactivity score (IRS) for LC3 expression. The staining results were scored based on the following criteria: (a) percentage of positive tumor cells in the tumor tissue: zero (0%), 1 (1%-25%), 2 (26%-50%), 3 (51%-75%), and 4 (76%-100%); (b) signal intensity: zero (no signal), 1 (weak), 2 (moderate), 3 (marked). IRS was calculated by multiplying the score for the percentage of positive cells by the intensity score (range of 0 to 12). The average IRS for each case was assigned as the staining result for the patient. The specimens were rescored if the difference between the scores determined by the two pathologists was greater than 3 [25]. The median IRS was defined as the cut-off value; IRS greater than this value was considered high and, otherwise, low.

Statistical analysis

Statistical analysis was performed using SPSS software (standard version 16.0, SPSS, Chicago, IL, USA). The correlation between LC3 expression and clinicopathological features was assessed using Pearson's χ^2 test. Disease-free survival (DFS) was defined as the time from surgery to regional relapse or distant metastasis. Overall survival (OS) was defined as the time from surgery to death. DFS and OS were assessed using the Kaplan-Meier method and compared by the log-rank test. Multivariate survival analysis was performed for all of the parameters that were significant in the univariate analysis using the Cox regression model. A two-sided probability value of less than 0.05 was considered statistically significant.

Results

Patient characteristics

Seventy females and one-hundred and eighty-three males, aged from 32 to 80 years (median
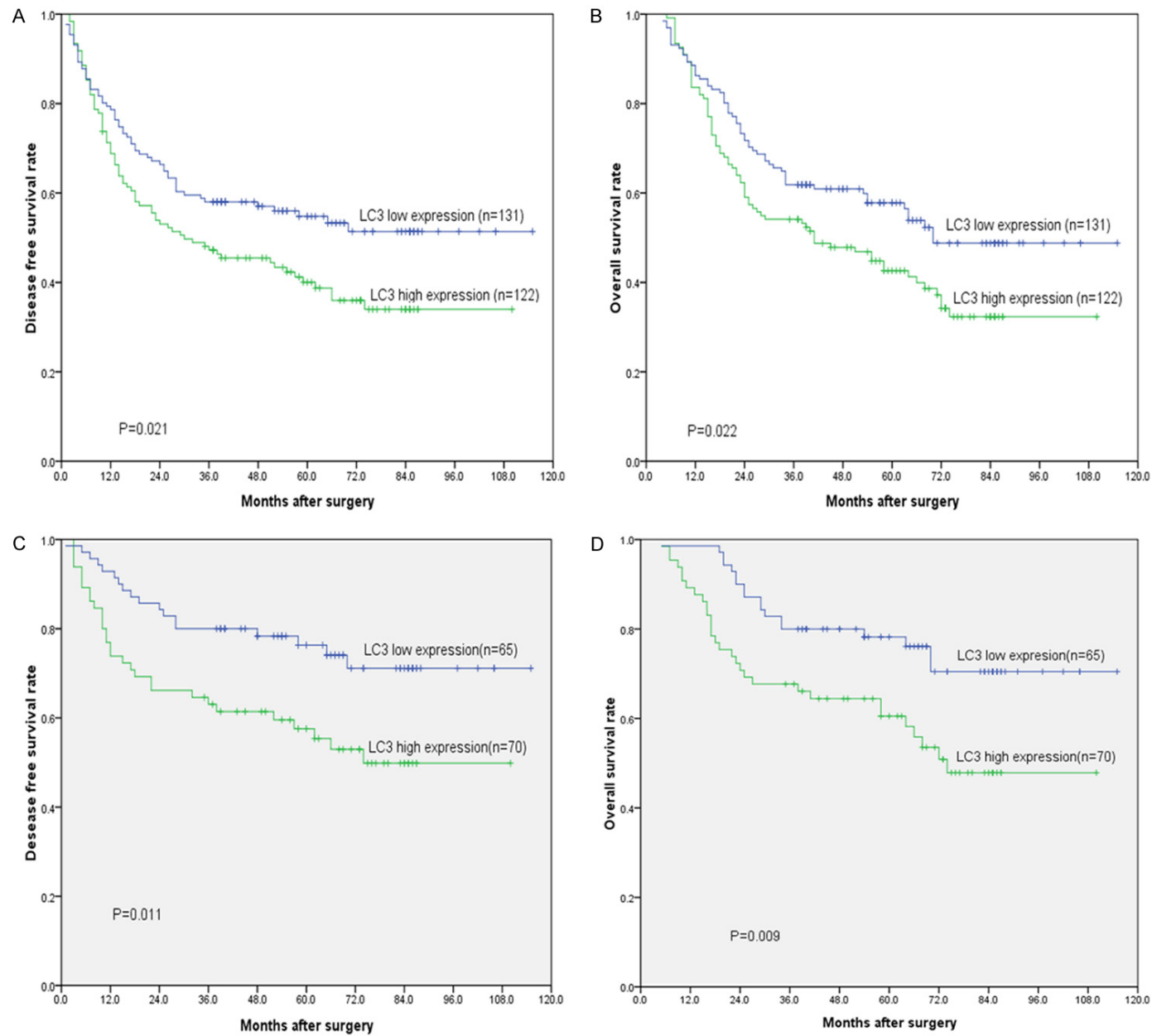
Figure 2. Disease-free survival (DFS) and overall survival (OS) curves for esophageal squamous cell carcinoma patients according to LC3 expression status. A, B: DFS and OS curves: patients with low and high expression levels of LC3. C, D: DFS and OS curves: patients with low and high expression levels of LC3 at stage N0.

Table 2. Kaplan-Meier survival analysis (log-rank test) according to LC3 expression in ESCC patients

Variable	Case number	DFS (months)			OS (months)		
		Mean	Median	*P*-value	Mean	Median	*P*-value
Total							
Low expression	131	68.7	NR	0.021	71.1	70.0	0.022
High expression	122	51.8	30.0		55.5	40.0	
pT1-2							
Low expression	37	68.2	NR	0.194	70.9	NR	0.243
High expression	28	57.3	57.0		62.1	66.0	
pT3-4							
Low expression	94	66.3	NR	0.074	68.7	70.0	0.061
High expression	94	42.6	26.0		46.3	39.0	
pN0							
Low expression	70	90.4	NR	0.011	92.1	NR	0.009
High expression	65	67.2	74.0		70.0	74.0	
pN1-3							
Low expression	61	37.3	18.0	0.515	39.6	26.0	0.597
High expression	57	29.7	16.0		35.0	24.0	
Histologic grade							
G1							
Low expression	32	63.8	70.0	0.447	66.0	70.0	0.540
High expression	26	59.5	51.0		63.0	64.0	
G2-3							
Low expression	99	68.1	NR	0.033	70.6	70.0	0.029
High expression	96	42.5	26.0		46.5	39.0	

ESCC: esophageal squamous cell carcinoma; DFS: disease-free survival; OS: overall survival; NR: not reached.

58.0 years), were included in the study. The clinicopathological characteristics of the 253 patients are listed in **Table 1**.

Expression of LC3 in ESCC

In the present study, LC3 staining of ESCC tissue and normal esophageal mucosa revealed immunoreactivity primarily in cytoplasm within tumor cells. The protein expression of LC3 was examined by IHC in 253 cases of primary ESCC and in 56 cases of paracancerous esophageal mucosa. Using the criteria described above (median IRS of 8.0), high expression of LC3 was observed in 62.5% (122/253) of the ESCC. There was no significant correlation of LC3 expression with clinicopathological parameters, such as age, sex, tumor location, histologic grade, T status, N status and pathological stage.

LC3 expression and survival

Among the 253 ESCC patients, no patients were lost to follow-up. The median observation period was 63.0 months (4-115 months), and 135 patients were deceased and 118 were alive at the end of the follow-up. The 5-year DFS and OS for the entire cohort were 47.5% and 50.3%, with median survival times of 52.0 and 63.0 months, respectively.

Patients with low expression of LC3 demonstrated longer OS compared with those with high expression of LC3 (mean of 71.1 months versus 55.5 months, *P* = 0.022, **Figure 2**, **Table 3**). A similar result was obtained for DFS (mean of 68.7 months versus 51.8 months, *P* = 0.021, **Figure 2**, **Table 2**). In the subgroup analysis, LC3 expression distinguished the DFS/OS well for pathological N0 patients (**Table 2**, *P* = 0.011/0.009), but not for pathological N1-3 patients (**Table 2**, *P* = 0.515/0.597).

Univariate analysis using Cox's proportional hazard model showed that the following parameters correlated significantly with DFS and OS: T category, N category, and LC3 expression (**Table 2**). When the above parameters were included in multivariate analysis, the results suggested that T category, N category, and LC3 expression were independent factors that affected OS (**Table 3**).

Discussion

LC3 is an autophagasomal orthologue of yeast autophagy-related gene 8 (Atg8), introduction of autophagy by various stresses such as starvation, hypoxia, stimulates up-regulation of LC3 expression. In order to investigate the role of LC3 in ESCC, we evaluated LC3 expression in ESCC tissues using high throughput tissue microarray. Consistent with studies in several other tumor entities, including esophageal

Table 3. Results of the univariate and multivariate survival analyses for OS according to the Cox regression model

	RR	95% CI	*P*-value
Univariate survival analysis			
Age (≤60 vs. >60)	1.142	0.812-1.606	0.444
Gender (male vs. female)	1.262	0.858-1.855	0.237
Tumor location[g]	0.909	0.649-1.273	0.579
Surgery (left thoracotomy vs. three incision)	0.142	0.812-1.606	0.444
Histologic grade[h]	1.297	0.971-1.731	0.078
T category[i]	1.470	1.010-2.140	0.044
N category (0 vs. 1/2/3)	3.465	2.414-4.972	<0.001
LC3 expression (high vs. low)	1.479	1.053-2.077	0.024
Multivariate survival analysis			
T category	1.389	0.963-2.005	0.079
N category (1/2/3 vs. 0)	3.388	2.358-4.868	<0.001
LC3 expression (high vs. low)	1.407	1.001-1.977	0.049

[g]Tumor location: upper vs. middle vs. lower; [h]Histologic grade: G1 vs. G2 vs. G3; [i]T category: T1 vs. T2 vs. T3 vs. T4; RR: relative risk; CI: confidence interval.

squamous cell carcinoma, gastric cancer and colorectal cancer [23], our results showed that a significant percentage of cells in the esophageal cancer mucosa demonstrated positive staining for LC3 compared with those in noncancerous esophageal mucosa. This may due to basal autophagy plays an important role in maintaining homeostasis in normal tissue [26, 27].

Increasing evidence indicates that autophagy plays an important role in cancer development. LC3, as a specific molecular biomarker of autophagy, also has been involved in carcinogenesis [28, 29]. In the present study, no significant correlation was observed between clinicopathological parameters and LC3 expression statistically. Nevertheless, high expression of LC3 in ESCC has shown shorter survival than the ones of low expression. Similar results were also reported in melanoma [29]. The lack of prognostic significance of LC3 was also reported in other surgical series of the patients with ESCC [23], this discrepancy is not surprising in light of studies with the difference of the sample enrolled.

Surgical resection can be considered as the standard treatment for patients with local ESCC. However, the problem how to identify the patients who could benefit from surgery is still unresovled. In the present study, elevated expression of LC3 was found to be an unfavorable prognostic factor in ESCC patients. High expression of LC3 was one of the most important predictors of poor DFS and OS in the multivariate analysis. This result was similar to the previous study reported on melanoma [29]. Therefore, we could conclude that LC3 is closely correlated with clinical outcome in human ESCC.

How LC3 promotes progression of ESCC is elusive. One possibility may that LC3 upregulation may represent an adaptive cellular mechanism directed to overcome uncontrolled proliferation and metabolic stress such as hypoxia and nutrient deprivation. Another possible mechanism is that the relatively poor blood supply in esophageal mucosa, increased expression of LC3 in cancer cells is more likely to sustain survival at this situation [30]. The third potential mechanism may relate to activation of positive regulator of apoptosis such as Bcl-2/ induced autophagy [31]. In future, identification the underlined mechanism would be helpful to designing ESCC patient-tailored therapy.

LC3 expression could be used to stratify DFS and OS in different subsets of patients, especially in pN0 stage patients, but not in stage pN1-3. This finding was supported by the previous study in ESCC [23], which suggest that LC3 is closely associated with the early phase of tumorigenesis in ESCC, but not with advanced stage. Therefore, the determination of LC3 expression by IHC could be used for these patients, who are more likely to experience disease recurrence or progression after surgical resection. In addition, adjuvant therapies should be recommended to these patients with higher expression of LC3 in pN0 stage.

There are some limitations in this study. Firstly, the samples selected from a single institution, and the number of samples enrolled may not enough for subgroup analysis. Subsequently, this is a retrospective study. Last, the information on chemotherapy or radiotherapy is inadequate to draw a conclusion about the potential role of LC3 expression to therapeutic sensitivity.

In conclusion, the present study determined the prognostic value of LC3 in the ESCC patients treated with surgical resection. LC3, as detected by IHC, may serve as a novel molecular marker for the prognosis of ESCC patients treated with surgical resection. Further studies are needed to evaluate the role of LC3 and clinical application in the treatment of ESCC.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Xin Wang, Department of Thoracic Surgery, Sun Yat-Sen University Cancer Center, 651 Dongfeng East Road, Guangzhou 510060, Guangdong Province, P.R. China. Tel: +86-20-87343596; E-mail: wangxin@sysucc.org.cn

References

[1] Jemal A, Siegel R, Xu J and Ward E. Cancer statistics, 2010. CA Cancer J Clin 2010; 60: 277-300.

[2] Hongo M, Nagasaki Y and Shoji T. Epidemiology of esophageal cancer: Orient to Occident. Effects of chronology, geography and ethnicity. J Gastroenterol Hepatol 2009; 24: 729-735.

[3] Tepper J, Krasna MJ, Niedzwiecki D, Hollis D, Reed CE, Goldberg R, Kiel K, Willett C, Sugarbaker D and Mayer R. Phase III trial of trimodality therapy with cisplatin, fluorouracil, radiotherapy, and surgery compared with surgery alone for esophageal cancer: CALGB 9781. J Clin Oncol 2008; 26: 1086-1092.

[4] Enzinger PC and Mayer RJ. Esophageal cancer. N Engl J Med 2003; 349: 2241-2252.

[5] Hoshino M, Fukui H, Ono Y, Sekikawa A, Ichikawa K, Tomita S, Imai Y, Imura J, Hiraishi H and Fujimori T. Nuclear expression of phosphorylated EGFR is associated with poor prognosis of patients with esophageal squamous cell carcinoma. Pathobiology 2007; 74: 15-21.

[6] Mann SS and Hammarback JA. Molecular characterization of light chain 3. A microtubule binding subunit of MAP1A and MAP1B. J Biol Chem 1994; 269: 11492-11497.

[7] Tanida I, Ueno T and Kominami E. LC3 conjugation system in mammalian autophagy. Int J Biochem Cell Biol 2004; 36: 2503-2518.

[8] Hemelaar J, Lelyveld VS, Kessler BM and Ploegh HL. A single protease, Apg4B, is specific for the autophagy-related ubiquitin-like proteins GATE-16, MAP1-LC3, GABARAP, and Apg8L. J Biol Chem 2003; 278: 51841-51850.

[9] Mizushima N, Yamamoto A, Matsui M, Yoshimori T and Ohsumi Y. In vivo analysis of autophagy in response to nutrient starvation using transgenic mice expressing a fluorescent autophagosome marker. Mol Biol Cell 2004; 15: 1101-1111.

[10] Yue Z, Jin S, Yang C, Levine AJ and Heintz N. Beclin 1, an autophagy gene essential for early embryonic development, is a haploinsufficient tumor suppressor. Proc Natl Acad Sci U S A 2003; 100: 15077-15082.

[11] Kanzawa T, Kondo Y, Ito H, Kondo S and Germano I. Induction of autophagic cell death in malignant glioma cells by arsenic trioxide. Cancer Res 2003; 63: 2103-2108.

[12] Liang XH, Jackson S, Seaman M, Brown K, Kempkes B, Hibshoosh H and Levine B. Induction of autophagy and inhibition of tumorigenesis by beclin 1. Nature 1999; 402: 672-676.

[13] Miracco C, Cosci E, Oliveri G, Luzi P, Pacenti L, Monciatti I, Mannucci S, De Nisi MC, Toscano M, Malagnino V, Falzarano SM, Pirtoli L and Tosi P. Protein and mRNA expression of autophagy gene Beclin 1 in human brain tumours. Int J Oncol 2007; 30: 429-436.

[14] Ahn CH, Jeong EG, Lee JW, Kim MS, Kim SH, Kim SS, Yoo NJ and Lee SH. Expression of beclin-1, an autophagy-related protein, in gastric and colorectal cancers. APMIS 2007; 115: 1344-1349.

[15] Chen Y, Lu Y, Lu C and Zhang L. Beclin-1 expression is a predictor of clinical outcome in patients with esophageal squamous cell carcinoma and correlated to hypoxia-inducible factor (HIF)-1alpha expression. Pathol Oncol Res 2009; 15: 487-493.

[16] Kato K, Ogura T, Kishimoto A, Minegishi Y, Nakajima N, Miyazaki M and Esumi H. Critical roles of AMP-activated protein kinase in constitutive tolerance of cancer cells to nutrient deprivation and tumor formation. Oncogene 2002; 21: 6082-6090.

[17] Pouyssegur J, Dayan F and Mazure NM. Hypoxia signalling in cancer and approaches to enforce tumour regression. Nature 2006; 441: 437-443.

[18] Degenhardt K, Mathew R, Beaudoin B, Bray K, Anderson D, Chen G, Mukherjee C, Shi Y, Gelinas C, Fan Y, Nelson DA, Jin S and White E. Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis. Cancer Cell 2006; 10: 51-64.

[19] Cuervo AM. Autophagy: in sickness and in health. Trends Cell Biol 2004; 14: 70-77.

[20] Qadir MA, Kwok B, Dragowska WH, To KH, Le D, Bally MB and Gorski SM. Macroautophagy inhibition sensitizes tamoxifen-resistant breast cancer cells and enhances mitochondrial depolarization. Breast Cancer Res Treat 2008; 112: 389-403.

[21] Samaddar JS, Gaddy VT, Duplantier J, Thandavan SP, Shah M, Smith MJ, Browning D, Rawson J, Smith SB, Barrett JT and Schoenlein PV. A role for macroautophagy in protection against 4-hydroxytamoxifen-induced cell death and the development of antiestrogen resistance. Mol Cancer Ther 2008; 7: 2977-2987.

[22] Apel A, Herr I, Schwarz H, Rodemann HP and Mayer A. Blocked autophagy sensitizes resistant carcinoma cells to radiation therapy. Cancer Res 2008; 68: 1485-1494.

[23] Yoshioka A, Miyata H, Doki Y, Yamasaki M, Sohma I, Gotoh K, Takiguchi S, Fujiwara Y, Uchiyama Y and Monden M. LC3, an autophagosome marker, is highly expressed in gastrointestinal cancers. Int J Oncol 2008; 33: 461-468.

[24] Rice TW, Blackstone EH and Rusch VW. 7th edition of the AJCC Cancer Staging Manual: esophagus and esophagogastric junction. Ann Surg Oncol 2010; 17: 1721-1724.

[25] Rhodes A, Jasani B, Barnes DM, Bobrow LG and Miller KD. Reliability of immunohistochemical demonstration of oestrogen receptors in routine practice: interlaboratory variance in the sensitivity of detection and evaluation of scoring systems. J Clin Pathol 2000; 53: 125-130.

[26] Eskelinen EL and Saftig P. Autophagy: a lysosomal degradation pathway with a central role in health and disease. Biochim Biophys Acta 2009; 1793: 664-673.

[27] Mizushima N, Levine B, Cuervo AM and Klionsky DJ. Autophagy fights disease through cellular self-digestion. Nature 2008; 451: 1069-1075.

[28] DiPaola RS, Dvorzhinski D, Thalasila A, Garikapaty V, Doram D, May M, Bray K, Mathew R, Beaudoin B, Karp C, Stein M, Foran DJ and White E. Therapeutic starvation and autophagy in prostate cancer: a new paradigm for targeting metabolism in cancer therapy. Prostate 2008; 68: 1743-1752.

[29] Han C, Sun B, Wang W, Cai W, Lou D, Sun Y and Zhao X. Overexpression of microtubule-associated protein-1 light chain 3 is associated with melanoma metastasis and vasculogenic mimicry. Tohoku J Exp Med 2011; 223: 243-251.

[30] Mizushima N. Autophagy: process and function. Genes Dev 2007; 21: 2861-2873.

[31] Pattingre S, Tassa A, Qu X, Garuti R, Liang XH, Mizushima N, Packer M, Schneider MD and Levine B. Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. Cell 2005; 122: 927-939.