Int J Clin Exp Pathol 2016;9(3):3735-3742
www.ijcep.com /ISSN:1936-2625/IJCEP0015276

Original Article

Expression of autophagy-related proteins is associated with the clinical outcome of patients with advanced gastric cancer receiving fluoropyrimidine/platinum chemotherapy

Yanchang Xu[1*], Yisen Huang[2*], Guofeng Pan[1*], Zhixiong Li[1], Linwen Lin[1], Jian Chen[1], Jihuang Wu[1]

[1]Department of Gastrointestinal Surgery, The First Hospital of Putian City, Fujian Medical University, Putian, Fujian, China; [2]Department of Gastroenterology, The First Hospital of Quanzhou, Fujian Medical University, Putian, Fujian, China. [*]Equal contributors.

Received August 29, 2015; Accepted January 25, 2016; Epub March 1, 2016; Published March 15, 2016

Abstract: The precise prediction of the clinical outcome of cancer patients is crucial for determining therapeutic options and providing optimal cancer care. Although targeted therapy is widely used in cancer treatment, chemotherapy remains the first-line treatment for patients with advanced gastric cancer. This study aimed to investigate the expression of autophagy-related proteins, unc-51-like kinase 1 (ULK1) and beclin 1 (BECN1), in advanced gastric cancer to clarify their clinical significance in the prognosis assessment of patients receiving fluoropyrimidine/platinum chemotherapy. The expression levels of ULK1 and BECN1 in gastric cancer tissues from 149 patients with TNM stages III and IV were measured by immunohistochemical staining. Both ULK1 and BECN1 were upregulated in gastric cancer. High expression of ULK1 and BECN1 were associated with older age and poor differentiation, respectively. In the univariate survival analysis, ULK1 and BECN1 expression were identified as predictors of poor prognosis. Only BECN1 expression was independently associated with poor prognosis in multivariate analysis. This association was significantly evident in cases who were older than 65 years, male, never smokers, drinkers, and those with poor differentiation or TNM stage III. Furthermore, the combined analysis revealed a significant cumulative effect on overall survival. Taken together, the expression levels of autophagy-related proteins could predict clinical benefit of fluoropyrimidine/platinum chemotherapy in patients with advanced gastric cancer.

Keywords: Gastric cancer, autophagy, unc-51-like kinase 1, beclin 1, prognosis

Introduction

Gastric cancer is the fifth most common cancer and the third leading cause of death from cancer worldwide, with 951,600 new cases diagnosed and 723,100 deaths occurred in 2012 [1]. Chronic infection with Helicobacter pylori is the strongest known risk factor for gastric cancer [2]. In China, Gastric cancer remains the third leading cancer diagnosis due to high prevalence of Helicobacter pylori infection [3, 4]. Despite the success of targeted therapies in some types of cancer, only few targeted therapies are available to treat gastric cancer. Chemotherapy remains the first-choice treatment for the majority of patients with gastric cancer.

Autophagy has been known to play an important role in adaptive responses to stress such as starvation and drug treatment, and is therefore necessary for cell survival under stress [5-7]. However, in other cases autophagy promotes cell death [5, 8]. Continued excessive or insufficient autophagic activity is involved in various human diseases, including cancer and Parkinson's disease [5, 7, 8]. The role of autophagy in cancer is extremely complex and remains less clear. It is most likely that autophagy probably has a preventive effect against cancer initiation, but facilitates cancer cell growth, proliferation and survival once a tumor develops [7]. Increasing evidence has demonstrated that cancer cells resistance to anticancer therapies including radiation therapy, chemotherapy and

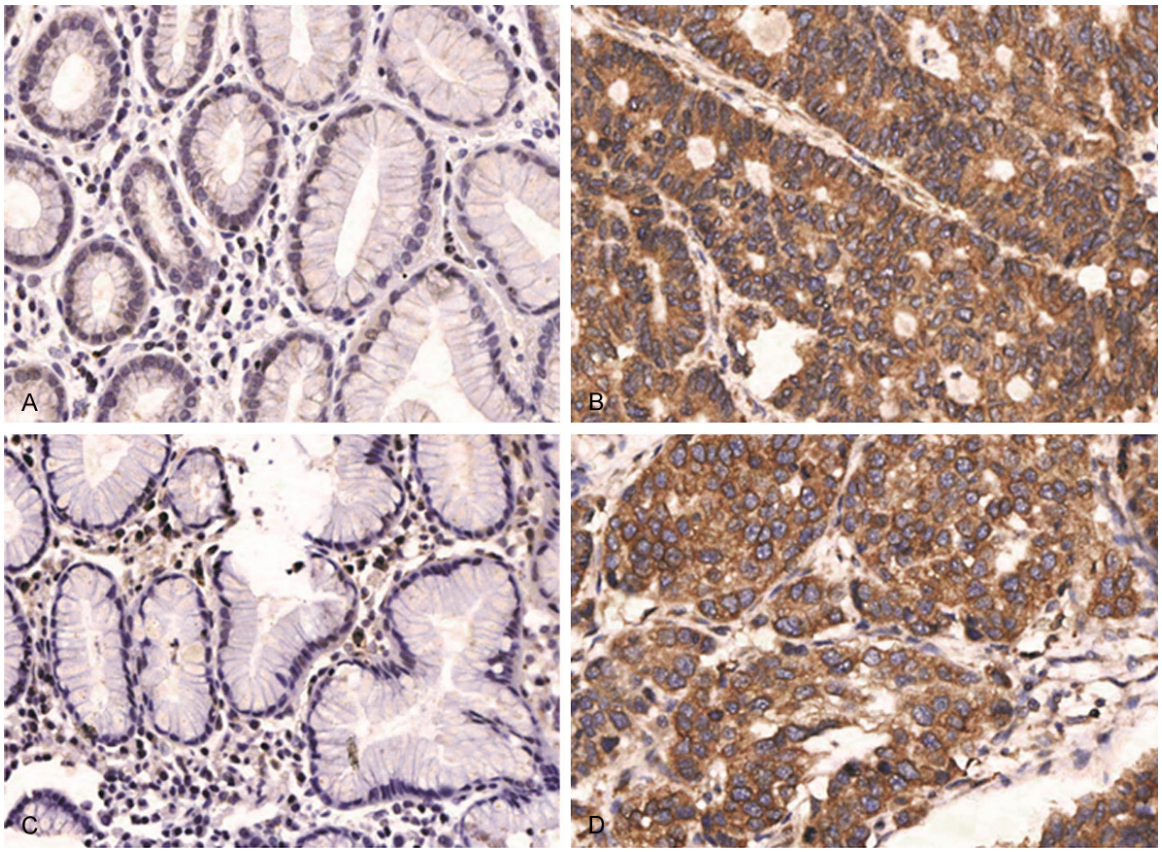

Figure 1. Immunohistochemical analysis of ULK1 and BECN1 in gastric cancer and adjacent non-cancerous tissues. ULK1 showed negative expression in paracancerous tissues (A) and strong positive expression in gastric cancer tissues (B). BECN1 showed negative expression in paracancerous tissues (C) and strong positive expression in gastric cancer tissues (D).

targeted therapy have more higher basic autophagic activity [9-11]. Therefore, disruption of autophagy can enhance the effectiveness of anticancer drugs [9, 12-14]. Many trials were performed to investigate the effectiveness of anticancer drugs in combination with autophagy inhibitors, such as chloroquine (CQ) and its derivative hydroxychloroquine (HCQ), for treating cancer (http://clinicaltrials.gov/).

Autophagy is controlled and coordinated by over 34 autophagy-related proteins. Inhibition of autophagy-related proteins can reduce cancer cell viability and increase sensitivity of cancer cells to anti-cancer drugs [13, 14]. Aberrant expression of autophagy-related proteins is associated with prognosis of various types of cancer, including gastric, esophageal, and colorectal cancers [8, 15-21]. However, whether autophagy affects the prognosis of gastric cancer patients receiving fluoropyrimidine/platinum chemotherapy remains unknown. To clarify this issue, we investigated the expression levels of unc-51-like kinase 1 (ULK1) and beclin 1 (BECN1) in gastric cancer tissues, and evaluated the relationship between ULK1 and BECN1 expression and the efficacy of fluoropyrimidine/platinum chemotherapy in patients with advanced gastric cancer.

Materials and methods

Patients

Gastric cancer and adjacent noncancerous tissues from 149 patients were collected from Hospital. All patients had histologically confirmed advanced-stage (stage III or IV) gastric adenocarcinoma, with a median age of 62 years (range: 28-84 years). Patients were excluded from the study if they had a history of cancer. Of 149 patients, 104 (69.8%) were male, and 66 (44.3%) were at TNM stage IV. All patients signed informed consent for the use of their tissues in this study according to the protocol approved by the Ethics Committee of the First Hospital of Putian City.

Table 1. Association of BECN1 and ULK1 expression with clinicopathologic parameters

Clinicopathologic parameters	BECN1		*P* value	ULK1		*P* value
	Positive	Negative		High	Low	
Age (years)						
> 65	30 (47.6)	33 (52.4)	0.184	38 (60.3)	25 (39.7)	0.046
≤ 65	51 (59.3)	35 (40.7)		66 (76.7)	20 (23.3)	
Sex						
Male	57 (54.8)	47 (45.2)	1.00	71 (68.3)	33 (31.7)	0.567
Female	24 (53.3)	21 (46.7)		33 (73.3)	12 (26.7)	
Histologic grade						
2	23 (71.9)	9 (28.1)	0.028	20 (62.5)	12 (37.5)	0.385
3	58 (49.6)	59 (50.4)		84 (71.8)	33 (28.2)	
Smoking status						
Smoker	41 (62.1)	25 (37.9)	0.100	47 (71.2)	19 (28.8)	0.858
Non-smoker	40 (48.2)	43 (51.8)		57 (68.7)	26 (31.3)	
Drinking status						
Drinker	56 (50.9)	54 (49.1)	0.191	78 (70.9)	32 (29.1)	0.686
Non-drinker	25 (64.1)	14 (35.9)		26 (66.7)	13 (33.3)	
TNM stage						
III	42 (50.6)	41 (49.4)	0.324	56 (67.5)	27 (32.5)	0.590
IV	39 (59.1)	27 (40.9)		48 (72.7)	18 (27.3)	

The chemotherapeutic regimen was doublet chemotherapy containing fluoropyrimidine (5-FU or capecitabine) and platinum (cisplatin or oxaliplatin). All patients were treated with at least two cycles of chemotherapy. The primary endpoint was overall survival which was defined as the time from the date of diagnosis to the death due to any cause or the last follow-up, and censored at the last observation date that each patient was known to be alive. The median follow-up duration was 31.0 months. During 78 months follow-up, 108 (72.5%) patients were died, with the five-year survival rate of 27.3%.

Immunohistochemistry (IHC) assay

For IHC staining, 4-micrometer-thick sections were deparaffinized, and endogenous peroxidase was blocked by 3% H_2O_2. After antigen retrieval, sections were treated with monoclonal antibody against ULK1 and BECN1, respectively, and then with secondary antibody. Furthermore, a known ULK1 positive case and ABECN1 positive cases were used as the positive controls, and phosphate buffered saline was used as negative control.

The expression levels of ULK1 and BECN1 were independently evaluated by two pathologists who were unware of the clinical data. All cases were scored using a semi-quantitative scoring method, as described previously [22]. To evaluate the relationship between the expression levels of ULK1 and BECN1 and clinical features and overall survival, patients were divided into high or low expression group according to the reference [22].

Statistical analyses

Statistical analyses were performed using SPSS soft-ware (version 20.0, SPSS Inc. Chicago, IL). Associations between ULK1 and BECN1 expression and clinical features were examined using χ^2 test. The Kaplan-Meier model was used to estimate the survival rates, and differences in survival between subgroups were compared using log-rank test. Cox proportional hazards regression model was used to examine the associations of clinical and pathologic variables, such as age, sex, tumor differentiation, smoking status, drinking status, and TNM stage, ULK1, and BECN1, and overall survival. A two-sided *P* value of < 0.05 was considered statistically significant.

Results

ULK1 and BECN1 expression in gastric cancer

Representative images of negative and positive ULK1 and BECN1 immunostains were shown in **Figure 1**. Specific staining of ULK1 and BECN1 was mostly found in the cytoplasm. Both ULK1 and BLCN1 were significantly upregulated in gastric cancer tissues compared with adjacent non-cancerous tissues ($P < 0.001$).

Association of ULK1 and BECN1 expression with clinicopathological features

The descriptive statistics of gastric cancer samples were presented in **Table 1**, by the expression status of ULK1 and BECN1, respec-

Table 2. Univariate and multivariate Cox regression analysis of overall survival in 168 patients with advanced gastric cancer

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age (≤ 65 years vs > 65 years)	1.300 (0.883-1.915)	0.184	1.351 (0.897-2.033)	0.149
Sex (male vs female)	1.032 (0.685-1.555)	0.880	1.007 (0.662-1.531)	0.974
Histologic grade (3 vs 2)	0.689 (0.445-1.067)	0.950	0.656 (0.415-1.037)	0.071
Smoking status (smoker vs non-smoker)	1.201 (0.817-1.764)	0.351	1.021 (0.682-1.529)	0.919
Drinking status (drinker vs non-drinker)	1.088 (0.714-1.658)	0.696	1.134 (0.736-1.747)	0.569
TNM stage (IV vs III)	1.534 (1.050-2.241)	0.027	1.706 (1.140-2.551)	0.009
BECN1 (high vs low)	0.593 (0.406-0.868)	0.007	0.560 (0.375-0.836)	0.005
ULK1 (high vs low)	1.545 (1.008-2.370)	0.046	1.332 (0.857-2.071)	0.203
Combination of BECN1 and ULK1 expression				
0	1		1	
1	1.909 (1.068-3.413)	0.029	1.894 (1.043-3.440)	0.036
2	2.523 (1.396-4.559)	0.002	2.633 (1.442-4.808)	0.002
0	1		1	
1+2	2.151 (1.242-3.727)	0.006	2.194 (1.251-3.847)	0.006

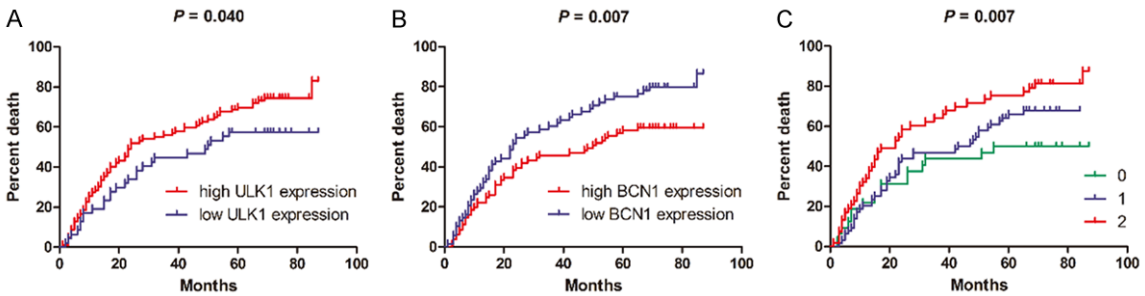

Figure 2. Kaplan-Meier curves of the overall survival of patients with advanced gastric cancer according to the expression levels of ULK1 and BECN1. Patients with low ULK1 expression (A) or high BECN1 expression (B) got better overall survival. The number of unfavorable factors (high ULK1 expression and low BECN1 expression) was positively correlated with the risk of mortality (C).

tively. ULK1 and BECN1 expression were significantly associated with age at diagnosis (*P* = 0.046) and histologic grade (*P* = 0.026), respectively. No significant association was observed between ULK1 and BECN1 expression and other clinicopathological features.

Effect of ULK1 and BECN1 expression on the overall survival of gastric cancer patients

In the univariate survival analysis, TNM stage (hazard ratio [HR] = 1.534, 95% confidence interval [CI]: 1.050-2.241, *P* = 0.027), ULK1 (HR = 1.545, 95% CI: 1.008-2.370, *P* = 0.046) and BECN1 expression (HR = 0.593, 95% CI: 0.406-0.868, *P* = 0.007) were associated with overall survival (**Table 2**). **Figure 2** presented the Kaplan-Meier overall survival curves for high and low ULK1 and BECN1 expression cases. In the multivariate survival analysis, only TNM stage (adjusted HR = 1.706, 95% CI: 1.140-2.551, *P* = 0.009) and BECN1 expression (adjusted HR = 0.560, 95% CI: 0.375-0.836, *P* = 0.005) remained significant after adjustment for age at diagnosis, sex, histological grade, smoking, and drinking status. Furthermore, we evaluated the cumulative effect of ULK1 and BECN1 expression on overall survival of gastric cancer patients. Patients with 1 (low BECN1 expression or high ULK1 expression) and 2 unfavorable factors (low BECN1 expression and high ULK1 expression) had 1.894- (95% CI: 1.043-3.440) and 2.633-fold (95% CI: 1.442-4.808) increased risk of death. There results indicated that patients

Table 3. Stratification analysis of GNL3 expression associated with survival of patients with gastric cancer

Variables	BECN1		ULK1	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age (years)				
> 65	0.389 (0.193-0.788)	0.009	1.178 (0.579-2.400)	0.651
≤ 65	0.636 (0.380-2.647)	0.084	1.437 (0.780-2.647)	0.245
Sex				
Male	0.577 (0.356-2.019)	0.025	1.197 (0.710-2.019)	0.500
Female	0.484 (0.211-1.113)	0.088	1.215 (0.499-2.961)	0.668
Histologic grade				
2	0.914 (0.330-2.537)	0.864	1.473 (0.583-3.724)	0.413
3	0.490 (0.308-0.782)	0.003	1.458 (0.853-2.492)	0.168
Smoking				
Smoker	0.586 (0.315-1.090)	0.091	1.199 (0.587-2.450)	0.619
Never	0.545 (0.318-0.934)	0.027	1.352 (0.740-2.470)	0.327
Alcohol drinking				
Drinker	0.495 (0.309-0.792)	0.003	1.170 (0.692-1.978)	0.557
Never	0.964 (0.394-2.361)	0.937	1.796 (0.736-4.383)	0.198
TNM stage				
III	0.489 (0.282-0.850)	0.011	1.201 (0.647-2.228)	0.562
IV	0.635 (0.339-1.189)	0.156	1.443 (0.737-2.826)	0.285

with low BECN1 expression and/or high ULK1 expression could not benefit from fluoropyrimidine/platinum chemotherapy.

Stratified analyses

To exactly investigate the effect of ULK1 and BECN1 expression on overall survival, we performed stratified analysis based on clinicopathological features. There were significant differences in overall survival between high and low BECN1 expression groups among patients older than 65 years, male patients, never smokers, drinkers, and those with poor differentiation or TNM stage III ($P < 0.05$, **Table 3**). However, stratified analyses based on clinicopathological features yielded non-significant association in approach to ULK1 expression ($P > 0.05$).

Discussion

Autophagy is the basic catabolic mechanism that involves degradation of unnecessary or dysfunctional cellular components through the resident lysosomal machinery [23]. In addition, autophagy serves prosurvival function in response to chemotherapeutic drugs in cancer cells, and thus suppression of autophagy during chemotherapy represents a novel therapeutic strategy for cancer [9, 12-14]. In the present study, we found that the expression levels of ULK1 and BECN1 were associated with the prognosis of advanced gastric cancer patients treated with fluoropyrimidine/platinum chemotherapy. Autophagy-related proteins may serve as biomarkers for identifying which patients will benefit from fluoropyrimidine/platinum chemotherapy.

BECN1, an essential regulator of autophagy, acts as a canonical initiator of autophagy through triggering a cascade of proteins involved in autophagolysosome formation [24, 25]. Mono-allelic deletion of BECN1 can promote tumor development [26]. Although BECN1 is downregulated in breast cancer [27], it is often upregulated in other types of cancer, including gastric [17], colorectal [15, 28], and liver cancers [29]. High expression level of BECN1 usually predict good prognosis in many types of cancer such as breast [27], gastric [17, 19], and liver cancers [29]. In agreement with the results of previous studies, we found that gastric cancer patients with high BECN1 expression had good prognosis when received fluoropyrimidine/platinum chemotherapy, especially for those with poor differentiation or drinkers. A study by Li et al. [30] showed that inhibition of BECN1 promoted autophagy in pancreatic cancer cells and decreased its sensitivity to gemcitabine. A recent study by Correa et al. revealed that BECN1 was dispensable for autophagy induction in ovarian cancer cells [31]. Apart from the role in triggering autophagy, it is far more important that BECN1 may delays cell cycle progression of cancer cells and induce differentiation once a tumor develops [32, 33]. Further studies are required to determine the precise mechanism underlying the role of BECN1 in gastric cancer.

ULK1 forms a stable complex with multiple proteins including ATG13 and FIP200, and this

complex is essential for the regulation of autophagy [34-37]. As a crucial autophagosomal modulating protein, ULK1 is often overexpressed in many types of human cancers, and may function as an oncogene to promote cancer cells growth, invasion, and metastasis [8, 16, 20, 38, 39]. In the early-stage cancer, ULK1 overexpression may contribute to promote autophagy initiation and protect cancer cells from apoptosis, especially for those at the early stage. Previous studies have demonstrated that high ULK1 expression is usually associated with poor prognosis of cancer patients [8, 16, 20, 38, 39]. In the present study, ULK1 was upregulated in gastric cancer, and its expression was associated with poor prognosis in gastric cancer patients. Although the difference disappeared after adjustment for age at diagnosis, sex, histological grade, smoking, and drinking, there was cumulative effect of ULK1 and BECN1 expression on the prognosis of gastric cancer patients. Combined use of ULK1 and BECN1 may improve the accuracy of predicting the prognosis of patients receiving chemotherapy. However, Tang et al. [21] reported that reduced expression of ULK1 was associated with decreased autophagic capacity in breast cancer, leading to disease progression. These findings imply different functions of ULK1 in different types of cancer.

In conclusion, our findings provide preliminary evidence for an association between autophagy-related proteins and outcome of patients with advanced gastric cancer receiving fluoropyrimidine/platinum chemotherapy. Examination of autophagy-related proteins might be helpful in choosing therapeutic options and determining the prognosis in patients with advanced gastric cancer. Further prospective studies are needed to confirm these findings.

Acknowledgements

This study was supported by Research Fund of the Putian Science and Technology Agency, Fujian, China [grant No. 2012 S01 (2)].

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Yanchang Xu, Department of Gastrointestinal Surgery, The First Hospital of Putian City, Fujian Medical University, Putian, Fujian, China. E-mail: cccxyc@163.com

References

[1] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.

[2] Plummer M, Franceschi S, Vignat J, Forman D and de Martel C. Global burden of gastric cancer attributable to Helicobacter pylori. Int J Cancer 2015; 136: 487-490.

[3] Chen W, Zheng R, Zeng H, Zhang S and He J. Annual report on status of cancer in China, 2011. Chin J Cancer Res 2015; 27: 2-12.

[4] Salih BA. Helicobacter pylori infection in developing countries: the burden for how long? Saudi J Gastroenterol 2009; 15: 201-207.

[5] Choi AM, Ryter SW and Levine B. Autophagy in human health and disease. N Engl J Med 2013; 368: 651-662.

[6] Rabinowitz JD and White E. Autophagy and metabolism. Science 2010; 330: 1344-1348.

[7] Jiang P and Mizushima N. Autophagy and human diseases. Cell Res 2014; 24: 69-79.

[8] Jiang L, Duan BS, Huang JX, Jiao X, Zhu XW, Sheng HH, Gao HJ and Yu H. Association of the expression of unc-51-Like kinase 1 with lymph node metastasis and survival in patients with esophageal squamous cell carcinoma. Int J Clin Exp Med 2014; 7: 1349-1354.

[9] Sui X, Chen R, Wang Z, Huang Z, Kong N, Zhang M, Han W, Lou F, Yang J, Zhang Q, Wang X, He C and Pan H. Autophagy and chemotherapy resistance: a promising therapeutic target for cancer treatment. Cell Death Dis 2013; 4: e838.

[10] Honscheid P, Datta K and Muders MH. Autophagy: detection, regulation and its role in cancer and therapy response. Int J Radiat Biol 2014; 90: 628-635.

[11] Jutten B and Rouschop KM. EGFR signaling and autophagy dependence for growth, survival, and therapy resistance. Cell Cycle 2014; 13: 42-51.

[12] Zhou J, Li G, Zheng Y, Shen HM, Hu X, Ming QL, Huang C, Li P and Gao N. A novel autophagy/mitophagy inhibitor liensinine sensitizes breast cancer cells to chemotherapy through DNM1L-mediated mitochondrial fission. Autophagy 2015; 11: 1259-1279.

[13] Zeng X, Zhao H, Li Y, Fan J, Sun Y, Wang S, Wang Z, Song P and Ju D. Targeting Hedgehog signaling pathway and autophagy overcomes drug resistance of BCR-ABL-positive chronic myeloid leukemia. Autophagy 2015; 11: 355-372.

[14] He J, Yu JJ, Xu Q, Wang L, Zheng JZ, Liu LZ and Jiang BH. Downregulation of ATG14 by EGR1-MIR152 sensitizes ovarian cancer cells to cisplatin-induced apoptosis by inhibiting cyto-protective autophagy. Autophagy 2015; 11: 373-384.

[15] Choi JH, Cho YS, Ko YH, Hong SU, Park JH and Lee MA. Absence of autophagy-related proteins expression is associated with poor prognosis in patients with colorectal adenocarcinoma. Gastroenterol Res Pract 2014; 2014: 179586.
[16] Jiang S, Li Y, Zhu YH, Wu XQ, Tang J, Li Z, Feng GK, Deng R, Li DD, Luo RZ, Zhang MF, Qin W, Wang X, Jia WH and Zhu XF. Intensive expression of UNC-51-like kinase 1 is a novel biomarker of poor prognosis in patients with esophageal squamous cell carcinoma. Cancer Sci 2011; 102: 1568-1575.
[17] Yu M, Gou WF, Zhao S, Xiao LJ, Mao XY, Xing YN, Takahashi H, Takano Y and Zheng HC. Beclin 1 expression is an independent prognostic factor for gastric carcinomas. Tumour Biol 2013; 34: 1071-1083.
[18] Geng QR, Xu DZ, He LJ, Lu JB, Zhou ZW, Zhan YQ and Lu Y. Beclin-1 expression is a significant predictor of survival in patients with lymph node-positive gastric cancer. PLoS One 2012; 7: e45968.
[19] Won KY, Kim GY, Lim SJ, Sung JY, Kim YW, Park YK, Lee J and Choi HS. Autophagy is related to the hedgehog signaling pathway in human gastric adenocarcinoma: prognostic significance of Beclin-1 and Gli2 expression in human gastric adenocarcinoma. Pathol Res Pract 2015; 211: 308-315.
[20] Liu B, Miyake H, Nishikawa M, Tei H and Fujisawa M. Expression Profile of Autophagy-related Markers in Localized Prostate Cancer: Correlation With Biochemical Recurrence After Radical Prostatectomy. Urology 2015; 85: 1424-1430.
[21] Tang J, Deng R, Luo RZ, Shen GP, Cai MY, Du ZM, Jiang S, Yang MT, Fu JH and Zhu XF. Low expression of ULK1 is associated with operable breast cancer progression and is an adverse prognostic marker of survival for patients. Breast Cancer Res Treat 2012; 134: 549-560.
[22] Zhang X, He C, He C, Chen B, Liu Y, Kong M, Wang C, Lin L, Dong Y and Sheng H. Nuclear PKM2 expression predicts poor prognosis in patients with esophageal squamous cell carcinoma. Pathol Res Pract 2013; 209: 510-515.
[23] Settembre C, Fraldi A, Medina DL and Ballabio A. Signals from the lysosome: a control centre for cellular clearance and energy metabolism. Nat Rev Mol Cell Biol 2013; 14: 283-296.
[24] 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.
[25] Mizushima N. The role of the Atg1/ULK1 complex in autophagy regulation. Curr Opin Cell Biol 2010; 22: 132-139.
[26] Qu X, Yu J, Bhagat G, Furuya N, Hibshoosh H, Troxel A, Rosen J, Eskelinen EL, Mizushima N, Ohsumi Y, Cattoretti G and Levine B. Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. J Clin Invest 2003; 112: 1809-1820.
[27] Tang H, Sebti S, Titone R, Zhou Y, Isidoro C, Ross TS, Hibshoosh H, Xiao G, Packer M, Xie Y and Levine B. Decreased mRNA Expression in Human Breast Cancer is Associated with Estrogen Receptor-Negative Subtypes and Poor Prognosis. EBioMedicine 2015; 2: 255-263.
[28] Wu S, Sun C, Tian D, Li Y, Gao X, He S and Li T. Expression and clinical significances of Beclin1, LC3 and mTOR in colorectal cancer. Int J Clin Exp Pathol 2015; 8: 3882-3891.
[29] Kang KF, Wang XW, Chen XW, Kang ZJ, Zhang X, Wilbur RR, Cheng F and Zhou SF. Beclin 1 and nuclear factor-kappaBp65 are upregulated in hepatocellular carcinoma. Oncol Lett 2013; 5: 1813-1818.
[30] Li X, Yan J, Wang L, Xiao F, Yang Y, Guo X and Wang H. Beclin1 inhibition promotes autophagy and decreases gemcitabine-induced apoptosis in Miapaca2 pancreatic cancer cells. Cancer Cell Int 2013; 13: 26.
[31] Correa RJ, Valdes YR, Shepherd TG and DiMattia GE. Beclin-1 expression is retained in high-grade serous ovarian cancer yet is not essential for autophagy induction in vitro. J Ovarian Res 2015; 8: 52.
[32] Koneri K, Goi T, Hirono Y, Katayama K and Yamaguchi A. Beclin 1 gene inhibits tumor growth in colon cancer cell lines. Anticancer Res 2007; 27: 1453-1457.
[33] Huerta S. Recent advances in the molecular diagnosis and prognosis of colorectal cancer. Expert Rev Mol Diagn 2008; 8: 277-288.
[34] Jung CH, Jun CB, Ro SH, Kim YM, Otto NM, Cao J, Kundu M and Kim DH. ULK-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery. Mol Biol Cell 2009; 20: 1992-2003.
[35] Ganley IG, Lam du H, Wang J, Ding X, Chen S and Jiang X. ULK1.ATG13.FIP200 complex mediates mTOR signaling and is essential for autophagy. J Biol Chem 2009; 284: 12297-12305.
[36] Hosokawa N, Hara T, Kaizuka T, Kishi C, Takamura A, Miura Y, Iemura S, Natsume T, Takehana K, Yamada N, Guan JL, Oshiro N and Mizushima N. Nutrient-dependent mTORC1 association with the ULK1-Atg13-FIP200 complex required for autophagy. Mol Biol Cell 2009; 20: 1981-1991.
[37] Lee JW, Park S, Takahashi Y and Wang HG. The association of AMPK with ULK1 regulates autophagy. PLoS One 2010; 5: e15394.

[38] Yun M, Bai HY, Zhang JX, Rong J, Weng HW, Zheng ZS, Xu Y, Tong ZT, Huang XX, Liao YJ, Mai SJ, Ye S and Xie D. ULK1: a promising biomarker in predicting poor prognosis and therapeutic response in human nasopharygeal carcinoma. PLoS One 2015; 10: e0117375.

[39] Xu H, Yu H, Zhang X, Shen X, Zhang K, Sheng H, Dai S and Gao H. UNC51-like kinase 1 as a potential prognostic biomarker for hepatocellular carcinoma. Int J Clin Exp Pathol 2013; 6: 711-717.