Association of Autophagy Defect with a Malignant Phenotype and Poor Prognosis of Hepatocellular Carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is an aggressive cancer with a poor prognosis. The role of autophagy and the prognostic value of autophagic genes are largely unknown in HCC. Here, we showed decreased expression of autophagic genes and their corresponding autophagic activity and increased expression of the antiapoptotic gene Bcl-xL in HCC cell lines compared with a normal hepatic cell line. We also found decreased expression of the autophagic gene Beclin 1 in 44 HCC tissue samples compared with adjacent nontumor tissues. In addition, we found that the most aggressive malignant HCC cell lines and HCC tissues with recurrent disease displayed much lower autophagic levels, especially when Bcl-xL was overexpressed. Interestingly, in a tissue microarray study consisting of 300 HCC patients who underwent curative resection, the expression of Beclin 1 was only significantly correlated with disease-free survival (DFS; \( P < 0.0001 \)) and overall survival (OS; \( P < 0.0001 \)) in the Bcl-xL+ group. Multivariate and univariate analyses also revealed that Beclin 1 expression was an independent predictor for DFS and OS in Bcl-xL+ patients. In addition, we found a significant correlation between Beclin 1 expression and tumor differentiation in Bcl-xL+ but not in Bcl-xL- HCC patients. In conclusion, our data showed expression of autophagic genes and their corresponding autophagic activities were suppressed in HCC. The autophagy defects synergized with altered apoptotic activity might facilitate tumor malignant differentiation, which results in a more aggressive cancer cell phenotype and poor prognosis of HCC. [Cancer Res 2008; 68(22):9167–75]

Introduction

Hepatocellular carcinoma (HCC) is one of the most frequent malignant tumors in southern China, Taiwan, southeastern Asia, and sub-Saharan Africa (1). The overall survival (OS) of HCC patients remains poor despite improved diagnostic and treatment strategies. Indeed, HCC is a type of cancer highly resistant to conventional antineoplastic medicines (2), which is partially attributed to the property of insensitivity to cell death induced by cytotoxic agents. It is well known that the avoidance of metabolic stress induced under starvation conditions by buffering metabolic stress induced under starvation conditions by recycling intracellular constituents (6). Although cell death resulting from progressive cellular consumption has been attributed to unrestrained autophagy, which results in the belief that autophagy is a nonapoptotic form of programmed cell death, most of the evidences support autophagy as a survival pathway required for cellular viability (7, 8). Interestingly, defects in autophagy also play a role in tumorogenesis. For example, Beclin 1, an important autophagy regulator Beclin 1 is monoallelically deleted in human ovarian, breast, and prostate cancers (9, 10). In addition, Beclin 1+/− or Atg4C−/− mice are prone to tumors (11–13). Paradoxically, these findings suggest that the loss of a survival pathway enhances tumor growth. Recent studies have shown that simultaneous defects in autophagy and apoptosis activate the DNA damage response in vitro, promote gene amplification and aneuploidy, and then accelerate mammary tumorigenesis (14, 15). Thus, loss of the prosurvival role of autophagy is likely to contribute to tumor progression by promoting genome damage and instability in an apoptosis-deficient background. Furthermore, stimulation of necrotic cell death and inflammation caused by defects in both autophagy and apoptosis provides a cell with nonautonomous means of tumor promotion through induction of a chronic wound-healing response (16). In fact, the protective role of autophagy that involves mitigation of genome damage and inflammation in tumors limits chronic tumor necrosis in response to metabolic stress and ultimately suppresses tumor carcinogenesis and progression. However, to date, the related clinical significance of this hypothesis has not been investigated.

Several molecules involved in the control and execution steps of autophagy have highlighted the close link among the autophagy, tumorigenesis, and tumor progression. Although Beclin 1, an important autophagy regulator, has been found to be monoallelically deleted or express at decreased levels in some human cancers, its expression pattern in HCC and the role of such molecules in clinical prognosis are largely unknown.

To study the role of autophagy in HCC under different apoptotic conditions, we examined the expression of autophagic genes, their corresponding autophagic activity, and expression of antiapoptotic genes in HCC cell lines and tissue samples. Our results suggest that in HCC with compromised apoptosis, autophagy defects are not only associated with malignant phenotype and poor differentiation...
of HCC cells but also represent poor survival, which can be independently predicted by the autophagic gene Beclin 1.

Materials and Methods

Patient samples. Patient samples were collected after obtaining informed consent according to an established protocol approved by the Ethics Committee of Fudan University. The data do not contain any information that may lead to the identification of the patients. Samples used in real-time PCR studies were randomly collected from the patients undergoing curative resection at the Liver Cancer Institute, Zhongshan Hospital, Fudan University, in March 2006. Samples were collected immediately after resection, transported in liquid nitrogen, and stored at −80°C. Sixteen frozen tissue samples used in Western blotting studies were also obtained from above patients.

Tumor specimens used in tissue microarray (TMA) studies were obtained from 300 consecutive HCC patients who underwent curative resection without pretreatment at the Liver Cancer Institute, Zhongshan Hospital, Fudan University, between 1997 and 2000. For each patient, complete follow-up data were available and the diagnosis of HCC was confirmed by pathologic examination.

Cell lines. Human HCC cell lines, including HepG2, Hep3B, SMMC-7721, MHCC97-L (17–19), MHCC97-H (19), HCCLM3 (17, 20), HCCLM6 (21), and a normal human hepatic cell line, L-02 (22, 23), were routinely maintained in high-glucose DMEM or RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum, 100 units/mL penicillin, and 100 mg/mL streptomycin. All cell lines were cultured at 37°C in a humidified incubator in an atmosphere of 5% CO2.

Autophagy analysis. For starvation studies, cells were cultured on 0.1% gelatin-coated dishes or coverslips, washed twice with PBS, once with Earle's Balanced Salt Solution (EBSS; Sigma-Aldrich), then incubated in EBSS at 37°C for the indicated times. Autophagy was assessed by GFP-microtubule–associated protein light chain 3 (LC3) redistribution and electron microscopy (24).

For GFP-LC3 redistribution, L-02, SMMC-7721, and HCCLM3 cells were transfected with a GFP-LC3 expression plasmid using Lipofectamine 2000 (Invitrogen). Redistribution was detected 24 h after transfection using an inverted fluorescence microscope. The fraction of GFP-LC3-positive (>3 punctate staining sites per cell) cells was determined in three independent experiments. Eight random fields representing 200 cells were counted.

For electron microscopy, cells were immediately fixed with 2.5% glutaraldehyde (with 0.1 mol/L sodium cacodylate) and stored at 4°C until further processing. Cells were postfixed with 1% osmium tetroxide, followed by dehydration with graded alcohol series and propylene oxide. Samples were then embedded and ultrathin (50–60 nm) sections were cut using an ultramicrotome (LKB-I). Images were examined with a JEM-1200 EX electron microscope (JEOL, Tokyo, Japan).

Statistical analyses. Comparisons of quantitative data were analyzed using Student's t test between two groups or by one-way ANOVA for multiple groups. Categorical data were analyzed using the χ2 or Fisher's exact tests. The Kaplan-Meier method was used to determine survival probability and differences were assessed by the log-rank test. Cox univariate and multivariate regression analyses were used to determine independent prognostic factors. Statistical significance was set at P < 0.05. All analyses were performed using SPSS software (v.15.0).

Results

Autophagic genes expression, autophagic activity, and antiapoptotic genes expression in HCC cell lines. The mRNA and protein expression of autophagic genes (Atg5, Beclin 1, Atg7) was evaluated in several established HCC cell lines. Almost all autophagic genes were observed in lower mRNA expression levels compared with a normal hepatic cell line L-02 (Fig. 1A), which was paralleled by Western blotting at the protein level (Fig. 1B).

In those HCC cell lines, HepG2, Hep3B, SMMC-7721, and MHCC97-L cells have relatively normal morphologies and capabilities to secret plasma proteins (18) and relatively low invasiveness.
and metastatic potentials (17). MHCC97-H, HCCLM3, and HCCLM6 have been generated from a poor differentiated human HCC and have extremely high invasiveness and metastatic activities (17, 21). Therefore, they were separated into two groups consisting of either low-grade malignant HCC cells (HepG2, Hep3B, SMMC-7721, MHCC97-L) or high-grade malignant HCC cells (MHCC97-H, HCCLM3, HCCLM6).

Real-time PCR analysis showed that the mRNA expression levels of Atg5, Beclin 1, and Atg7 were lower in the high-grade malignant group than in the low-grade malignant group \( (P = 0.0003, P < 0.0001 \text{ and } P = 0.096, \text{ respectively}; \) Fig. 1A). Western blotting analysis also revealed decreased protein expression in the high-grade malignant group (Fig. 1B). These results suggested that decreased expression of autophagic genes might correlate with the malignant phenotype of HCC.

To examine the expression of antiapoptotic genes in HCC cell lines, the antiapoptotic Bcl-2 family proteins Bcl-2 and Bcl-xL were evaluated. As described previously (29), Bcl-2 expression was extremely low in the HCC cell lines (data not shown). In contrast, overexpression of Bcl-xL was observed in all HCC cell lines (Fig. 1B). Moreover, HCCLM3 was resistant to the apoptotic stimulator staurosporine, which can be reversed partially by Bcl-xL knockdown (Supplementary Fig. S1), suggesting that apoptosis resistance is a general characteristic of those HCC cell lines.

These initial observations indicated that autophagy may be down-regulated and antiapoptotic capability may be up-regulated in HCC cell lines.

To further confirm the relationship between autophagic activity and malignant phenotype in HCC, the autophagic activities of a normal hepatic cell line (L-02), a low-grade malignant HCC cell line (SMMC-7721), and a high-grade malignant HCC cell line (HCCLM3) were examined under starvation conditions. Electron microscopy revealed a low level of baseline autophagosome formation that was increased minimally in response to starvation treatment in

![Figure 1](cancerres.aacrjournals.org)
Autophagy Defect in HCC

Western blotting analysis of Beclin 1 expression in eight tumor tissue samples (four nonrecurrent and four recurrent cases) and corresponding nontumor tissue samples were confirmed by Western blot analysis on 8 HCC samples. Among above tumor samples, 9 cases decreased over 3-fold, and 11 cases exhibited 2-to-3-fold reductions. These findings were confirmed by Western blot analysis on 8 HCC samples selected from the 44 HCC cases. Decreased Beclin 1 protein levels were observed in most cases (6 of 8) especially in recurrent HCC compared with the nontumor tissues (Fig. 2A). Moreover, the lower Beclin 1 mRNA expression of HCC correlated with recurrent disease \( (P = 0.040) \), and poor differentiation of tumor was not significant \( (P = 0.183) \) (Fig. 2A). These findings, combined with the autophagic activity studies in cells, indicated that decreased Beclin 1 expression in HCC tissues might not only contribute to the autophagy defect but also influence the clinical prognosis.

As described earlier, the antiapoptotic protein Bcl-xL was overexpressed in a variety of HCC cell lines. However, no significant difference in Bcl-xL mRNA levels was observed between the recurrent and nonrecurrent groups in 44 HCC tissue samples \( (P = 0.708; \text{data not shown}) \), suggesting that decreased Beclin 1 expression rather than Bcl-xL overexpression might correlate with the poor prognosis of HCC.

To determine whether defects in apoptosis influence the autophagy-related prognosis, we divided the HCC cases into low Bcl-xL expression (Bcl-xL\(^-\)) and high Bcl-xL expression (Bcl-xL\(^+\)) groups by using the median Bcl-xL expression level as the cutoff. As shown in Fig. 2B, in the Bcl-xL\(^-\) group, the expression of Beclin 1 was significantly decreased in recurrent cases compared with nonrecurrent cases \( (P = 0.030) \). The mean relative Beclin 1 mRNA expression levels in recurrent and nonrecurrent cases were 0.028 and 0.060, respectively, in the Bcl-xL\(^-\) group, and 0.050 and 0.042, respectively, in the Bcl-xL\(^+\) group. Thus, we hypothesized that the autophagy-related prognosis of HCC might require antiapoptotic conditions.

We also examined the relationship between tumor differentiation and Beclin 1 expression. In the Bcl-xL\(^-\) group, the level of Beclin 1 expression was very similar in well-differentiated (0.049) and poorly differentiated (0.042) tumors \( (P = 0.742; \text{Fig. 2C}) \). However, in the Bcl-xL\(^+\) group, the mean relative Beclin 1 expression levels were 0.060 and 0.038, respectively \( (P = 0.105; \text{Fig. 2C}) \). These results indicated that autophagy might be correlated with tumor differentiation, especially under conditions in which apoptosis is compromised.

**Autophagy defect is associated with poor prognosis of HCC in Bcl-xL\(^-\)-positive background.** To further validate our proposed hypothesis, we used high-throughput TMA technology to assess the relationship between autophagy and prognosis of HCC in a group of 300 HCC patients who underwent curative resections. We found that 32% (95 of 300) and 52% (157 of 300) of the HCC patients exhibited positive Beclin 1 and Bcl-xL expression, respectively. Expression of both positive Beclin 1 and Bcl-xL was observed in 55 HCC cases (Fig. 3A).

The 3-, 5-, and 7-year disease-free survival (DFS) and OS rates of these HCC patients were 57.2% and 71.7%, 46.2% and 55.7%, and 41.5% and 43.9%, respectively. Patients with negative Beclin 1 expression had a significantly poorer prognosis than Beclin 1-positive patients (DFS, \( P = 0.0002; \text{OS, } P = 0.0022; \text{Fig. 3B} \)). The 3-, 5-, and 7-year DFS and OS rates for Beclin 1\(^-\) and Beclin 1\(^+\) patients were 51.6% and 69.8% versus 69.3% and 75.8%, 37.4% and 51.2% versus 65.8% and 65.3%, and 32.8% and 39.9% versus 60.6% and 52.5%, respectively.

When all HCC patients were stratified by Bcl-xL expression, we found that the prognosis of Beclin 1\(^-\) patients was much worse than for Beclin 1\(^+\) patients in the Bcl-xL\(^-\) group \( (P < 0.0001; \text{Fig. 3B}) \). However, no significant difference in the survival rates of Beclin 1\(^-\) and Beclin 1\(^+\) patients was observed in the Bcl-xL\(^+\) group (DFS, \( P = 0.978; \text{OS, } P = 0.233; \text{Fig. 3B} \)). In the Bcl-xL\(^-\) group, the 3-, 5-, and 7-year DFS and OS rates for Beclin 1\(^-\) and Beclin 1\(^+\) patients were 44.7% and 66.7% versus 81.7% and 85.5%, 25.8% and 43.1% versus 77.7% and 78.2%, and 22.3% and 30.2% versus 71.4% and 63.5%, respectively.

The prognostic value of Beclin 1 expression in HCC patients with a Bcl-xL\(^-\)-positive background. The correlation between Beclin 1 expression and clinicopathologic parameters in all HCC patients was statistically analyzed (Table 1). A significant correlation between the absence of Beclin 1 expression and high serum α-fetoprotein (AFP) levels was observed \( (P = 0.008) \). An absence of Beclin 1 expression occurred more frequently in poorly differentiated HCC than in well-differentiated HCC, although this difference was not statistically significant \( (P = 0.185) \). No significant correlation was found between Beclin 1 expression and other variables, including age, sex, tumor size, and tumor number.

Importantly, in the Bcl-xL\(^-\) group, a significant correlation between the absence of Beclin 1 and poor tumor differentiation

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**Figure 2.** Autophagic level in HCC tissue samples. A, relative Beclin 1 mRNA expression in nontumor and tumor tissue (top left), nonrecurrent and recurrent tumor tissue (top right), and well-differentiated and poorly differentiated tumor tissue (bottom left). Columns, mean from three independent experiments; bars, SD. Beclin 1 expression in eight tumor tissue samples (four nonrecurrent and four recurrent cases) and corresponding nontumor tissue samples were confirmed by Western blotting analysis (bottom right). B, a total of 44 HCC cases were divided into two groups—low Bcl-xL expression group (Bcl-xL\(^-\)) and high Bcl-xL expression group (Bcl-xL\(^+\)) by using the median of relative Bcl-xL mRNA expression as the cutoff. Relative Beclin 1 mRNA expression of nonrecurrent and recurrent tumor tissue in Bcl-xL\(^-\) group and Bcl-xL\(^+\) group. C, relative Beclin 1 mRNA expression of well-differentiated and poorly differentiated tumor tissue in the Bcl-xL\(^-\) and Bcl-xL\(^+\) groups.
was observed ($P = 0.033$). In contrast, this correlation was not statistically significant in the Bcl-xL$^-$ group ($P = 0.693$; Table 1).

The prognostic value of Beclin 1 expression was evaluated in HCC patients using univariate analysis, which showed that Bcl-xL expression, age, sex, HBV infection, liver cirrhosis, AFP levels, alanine aminotransferase levels, and tumor differentiation had no prognostic significance for DFS and OS. However, the tumor-node-metastasis stage, vascular invasion, tumor size, and tumor number were predictors for DFS and OS. Beclin 1 expression was also a significant predictor for tumor recurrence and OS ($P = 0.0003$ and $P = 0.023$, respectively) in all of the study population (Table 2). We further assessed the prognostic value of Beclin 1 in Bcl-xL$^+$ and Bcl-xL$^-$ HCC patients. Beclin 1 expression was a predictor for both DFS and OS in the Bcl-xL$^+$ group but not in the Bcl-xL$^-$ group (Supplementary Tables S1 and S2).

Multivariate analysis was conducted with four of the variables (Beclin 1 expression, vascular invasion, tumor size, and tumor number), which was shown to be significant in the univariate analysis and no obvious correlation between each other. Negative Beclin 1 expression was still the independent variable for predicting poor DFS and OS, especially in Bcl-xL$^+$ patients ($P < 0.0001$ and $P = 0.0002$, respectively; Supplementary Table S3).

Collectively, these results showed that in HCC with positive Bcl-xL expression, an autophagy defect (i.e., negative Beclin 1 expression) was associated with poor tumor differentiation and poor survival that could be independently predicted by the autophagic gene Beclin 1.

Discussion

Inactivation of autophagy-specific genes such as Beclin 1 has been shown to lead to increased tumorigenesis in mice. Enforced expression of such genes (Beclin 1 and Atg5) inhibits the formation of human breast tumors in mouse models (32). Furthermore, net deletions of several autophagy-specific genes are commonly found in human malignancies (32). Thus, autophagy may be a tumor-suppressor pathway and its decreased activity may contribute to the development of human cancer (33).

In the present study, we found that the expression of autophagic genes was extremely low in HCC cell lines especially in highly malignant HCC cell lines. Because mounting evidence shows that monoallelic deletion or decreased protein expression of Beclin 1 could lead to compromised autophagic activities both in vitro and in vivo (12, 14–16, 24, 30, 31, 34, 35), we...
focused on the autophagic gene Beclin 1. As a consequence, we examined its expression by real-time PCR in 44 HCC and adjacent nontumor tissues, and found that, consistent with breast cancer (9, 10), a significant decrease in Beclin 1 expression occurred in HCC tissues compared with nontumor tissues. These findings suggested that HCC might possess defective autophagy.

Antiapoptosis is also a characteristic of cancers that is regulated by a series of molecular cellular events (36). Members of the Bcl-2 protein family, key regulators of apoptosis, include antiapoptotic proteins such as Bcl-2 and Bcl-xL and proapoptotic proteins such as Bax and Bak (37). Deficiencies in proapoptotic proteins or expression of antiapoptotic proteins block apoptosis. Previous studies (29, 38, 39) have reported that in HCC, endogenous Bcl-xL inhibits apoptosis produced by various stress-inducing conditions such as staurosporine treatment, serum starvation, and p53 activation. The HCC cell lines established at our institute (such as MHCC97-L and HCCLM3) also exhibit resistance to a variety of apoptotic stimuli (data not shown). Here, we found that Bcl-xL is highly expressed in various HCC cells as previously reported (29) and the knockdown Bcl-xL can reverse the apoptotic resistance induced by staurosporine in HCCLM3 cells. Thus, we proposed that Bcl-xL may, at least partially, contribute to the antiapoptotic properties of HCC.

The functional relationship between autophagy and apoptosis within the tumor is complex. Initially, autophagy was considered to be an alternative pathway to cellular demise that was termed autophagic cell death (or type II cell death; ref. 40). However, inhibits apoptosis produced by various stress-inducing conditions such as staurosporine treatment, serum starvation, and p53 activation. The HCC cell lines established at our institute (such as MHCC97-L and HCCLM3) also exhibit resistance to a variety of apoptotic stimuli (data not shown). Here, we found that Bcl-xL is highly expressed in various HCC cells as previously reported (29) and the knockdown Bcl-xL can reverse the apoptotic resistance induced by staurosporine in HCCLM3 cells. Thus, we proposed that Bcl-xL may, at least partially, contribute to the antiapoptotic properties of HCC.

The functional relationship between autophagy and apoptosis within the tumor is complex. Initially, autophagy was considered to be an alternative pathway to cellular demise that was termed autophagic cell death (or type II cell death; ref. 40). However,

Table 1. Correlation between Beclin 1 expression and clinicopathologic characteristics in the whole study group and in the Bcl-xL–positive and Bcl-xL–negative groups

<table>
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<tr>
<th>Clinicopathologic Characteristics</th>
<th>Whole study group</th>
<th>Beclin1 expression</th>
<th>P</th>
<th>Bcl-xL–positive group</th>
<th>Beclin1 expression</th>
<th>P</th>
<th>Bcl-xL–negative group</th>
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<td>43</td>
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Abbreviations: ALT, alanine aminotransferase; TNM, tumor-node-metastasis; HBsAg, hepatitis B surface antigen.

*Fisher’s exact tests, and χ² tests for all the other analysis.
In contrast, in Bcl-xL expression level in well-differentiated tumors was higher than in the Bcl-xL+ group. Thus, our data suggest that the malignant correlation between Beclin 1 expression and tumor differentiation.

Chemical analysis of 300 HCC cases also revealed the significant well-differentiated cells did not differ significantly. Immunohistochemical analysis on 300 HCC tissue samples grouped according to their Bcl-xL expression revealed that the 3-, 5-, and 7-year OS and DFS rates in Beclin 1+ patients were significantly lower than in Beclin 1- patients. Moreover, Beclin 1 was the strongest independent predictor for OS and DFS in Bcl-xL+ HCC patients by both multivariate and univariate analyses. To our knowledge, this is the first report that describes an autophagy-related prognosis and shows the prognostic value of autophagic gene expression in human cancers.

In conclusion, our study shows that the expression of autophagic genes in high-grade malignant HCC cells compared with low-grade malignant ones. GFP-LC3 redistribution and electron microscopy analyses revealed that autophagic activity was suppressed in some HCC cell lines, especially in the more aggressive HCC cell lines. Our findings also confirmed that those specific HCC cells possessed compromised autophagic activity consistent with their decreased expression of autophagic genes. Moreover, those HCC cell lines all exhibited high Bcl-xL expression level, but we did not find a close correlation between Bcl-xL expression and HCC malignancy in these cells. Interestingly, when we evaluated Beclin 1 expression in HCC tissue samples grouped according to their Bcl-xL expression levels, we found that in Bcl-xL- HCC samples, the relative Beclin 1 expression level in well-differentiated tumors was higher than in poorly differentiated tumors. In contrast, in Bcl-xL+ HCC samples, the expression of Beclin 1 in poorly differentiated and well-differentiated cells did not differ significantly. Immunohistochemical analysis of 300 HCC cases also revealed the significant correlation between Beclin 1 expression and tumor differentiation in the Bcl-xL- group. Thus, our data suggest that the malignant phenotype or differentiation of HCC is only correlated closely with Beclin 1 expression in a Bcl-xL- positive background. These findings may indicate that an autophagy defect increases the accumulation of genome damage and mutation rate, ultimately promoting malignancy and invasive differentiation in HCC cells as described in previous studies.

An aggressive cancer cell phenotype always results in recurrence or a poor prognosis. Thus, this study, which focuses on the combined role of autophagy and apoptosis in the prognosis of HCC, is extremely important. We found that the relative Beclin 1 mRNA expression in a series of HCC patients with recurrent disease was significantly reduced compared with nonrecurrent cases in the Bcl-xL- group. Immunohistochemical analysis on Bcl-xL- HCC cases revealed that the 3-, 5-, and 7-year OS and DFS rates in Beclin 1- patients were significantly lower than in Beclin 1+ patients. Moreover, Beclin 1 was the strongest independent predictor for OS and DFS in Bcl-xL+ HCC patients by both multivariate and univariate analyses. To our knowledge, this is the first report that describes an autophagy-related prognosis and shows the prognostic value of autophagic gene expression in human cancers.

Our study raises the concern of why an autophagy defect was not associated with malignancy, invasive differentiation, and poor prognosis in Bcl-xL- negative HCC cells. We speculate that in most circumstances, tumor cells could be eliminated by apoptosis regardless of the functional status of autophagy. Moreover, several studies have shown that autophagic death only occurred when apoptosis was inhibited. Autophagy has previously been shown to play a survival role in the carcinogenesis and progression of tumors with intact apoptosis. Recently, it has been shown that concurrent inactivation of autophagy and apoptosis led to tumor inflammation and chronic necrosis that would promote tumorigenesis. On the other hand, complex mechanism is involved in regulating the malignant phenotype of HCC and there would be some alternative pathways independent of apoptosis or autophagy, or independent of being regulated by Bcl-xL and Beclin 1.

In conclusion, our study shows that the expression of autophagic genes and their corresponding autophagic activities are suppressed in some HCC cells. Autophagy defects may be associated with malignant phenotype and poor prognosis of HCC in an apoptosis-compromised background.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.
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References


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