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ORIGINAL ARTICLE

High expression of Beclin-1 predicts favorable prognosis for patients with colorectal cancer

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Summary
Purpose: Beclin-1 is an autophagy gene. It promotes the formation of the autophagic vesicle as well as plays an essential role in guarding the cells against chromosomal instability. Over-expression of Beclin-1 has been reported to predict a favorable survival in various cancers. However, little is known about its prognostic significance in colorectal cancer.
Methods and materials: A total of three hundred and sixty-three (363) colorectal tissues from colorectal cancer (CRC) patients were collected. Tissue micro-arrays and immunohistochemistry were used to investigate the expression and prognostic significance of Beclin-1 in CRC. The associations among Beclin-1 expression, clinicopathological parameters and prognosis were evaluated.
Results: Beclin-1 had a higher expression in CRC tissues than in normal tissues. A high expression of Beclin-1 was positively correlated with gender ($P = 0.027$), histological grade ($P = 0.003$), pM status ($P = 0.003$) and clinical stage ($P = 0.024$). Patients with a high Beclin-1 expression, when compared to those with a lower expression had both a better overall survival (OS, $P = 0.006$) and disease-free survival (DFS, $P = 0.008$). In the pT3 subgroup, Beclin-1 was also found to be a good prognostic indicator ($P < 0.05$). Multivariate analysis showed a high expression of Beclin-1 was indeed a positive independent prognostic factor of OS and DFS for CRC patients ($P < 0.05$).

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Conclusion: Our results demonstrated that a high expression of Beclin-1 correlated with a better overall survival and disease-free survival, thus serving as a favorable independent prognostic marker in CRC.

Introduction

CRC is a worldwide leading cause of cancer-related tumors [1]. Despite recent advancement, the 5-year survival rate for patients in the advanced stage has been reported to be less than 13% [2]. This is mainly due to either local recurrence and/or distant metastasis of the disease [3]. CRC prognosis is mainly predicted through tumor-node-metastasis (TNM) staging system. However, clinical studies have found that CRC patients having the same TNM stage have a substantial different prognosis, probably due to their varied molecular and genetic basis [4].

There is hence a crucial need to identify prognostic factors and independent biomarkers which would serve as reliable prognostic factors and complement the current TNM stage. Recently, molecular/genetic biomarkers have been shown to be more accurate than TNM staging. Since these biomarkers are directly linked to the molecular changes in cancer cells, they show a high sensitivity in predicting the prognosis and monitoring any relapse. For example, stage II CRC patients with a mismatch repair protein mutation have been shown to have a better prognosis [5]. Likewise, autophagy genes have shown much promise as potential biomarkers.

Autophagy, under stressful conditions such as starvation, provides essential amino acids and energy for protein synthesis, allowing the organism to survive [6]. Autophagy also regulates homeostasis in basically all cells, including cancer cells. Beclin-1, the first identified mammalian autophagy gene, has been mapped to a tumor-susceptibility locus on chromosome 17q21 [7]. The protein plays an essential role in tumor biology by decreasing the frequency of mutations, guarding the cells against chromosomal instability [8,9], and promoting the nucleation of the autophagic vesicle [10].

The particular autophagic effects of Beclin-1 protein have been associated to its interaction with several important cellular molecules, namely Bcl-2, Bcl-xL, HIF-1, mTOR, JNK, Ambra1, Bif-1, Vps34, Atg14 and UVRAG [5]. Recent studies have evaluated the prognostic significance of Beclin-1 in gastric cancer [11], laryngeal squamous cell carcinoma [12], ovarian carcinoma [13] and confirmed that Beclin-1 was indeed to be a useful prognostic biomarker.

In the present study, we addressed the prognostic value of Beclin-1 in 363 CRC patients who underwent radical surgery with R0 resection. We found a higher expression of Beclin-1 in tumor tissues than in paired normal tissues. The prognostic analysis showed that patients with a high expression of Beclin-1 had a superior OS ($P = 0.006$), and DFS ($P = 0.008$). We also found that a high expression of Beclin-1 was positively correlated with gender ($P = 0.027$), histological grade ($P = 0.003$), pM status ($P = 0.003$) and clinical stage ($P = 0.024$). Beclin-1 was found to be a good prognostic indicator in the pT3 subgroup (OS $P = 0.008$, DFS $P = 0.007$). As well, multivariate analysis confirmed that a high expression of Beclin-1 was a positive independent prognostic factor for CRC ($P < 0.05$).

Patients and methods

Patients' selection

Archival formalin-fixed-paraffin-embedded (FFPE) tumor tissues from 363 patients between January 2000 and December 2005 were obtained from tissues bank of Sun Yat-Sen University. No patients had received any neoadjuvant chemotherapy or chemoradiotherapy before the surgical resection. The detailed clinicopathological data is listed in Table 1. This study was approved by the Clinical Ethics Review Committee of Sun Yat-Sen University. All patients provided a written consent form.

Tissue micro-arrays (TMAs)

The TMAs were performed as we previously described [14]. We first re-reviewed the hematoxylin and eosin-stained slides. The tumor area for TMA design was selected right at the center of the tumor, avoiding areas of ulceration or necrosis. Three cores (1 mm in diameter) from the tumor area, along with an additional two cores (1 mm in diameter) taken from histologically confirmed normal adjacent colorectal mucosa were taken to construct the TMAs using Tissue Array (Alphelys, MINIPORE, Plaisir, France). Each core was regarded to represent a ''virtual biopsy'', assuming that the volume of the core represented by 3 tumor cores would roughly correspond to the tumor volume.

Immunohistochemistry (IHC) staining and evaluation

To detect Beclin-1 expression, rabbit polyclonal antibody against Beclin-1 was used (at a dilution of 1:100). The TMAs were sectioned at 4 μm intervals. To ensure tissue adhesion, the slides were incubated at 60 °C for 3 h. All sections were then deparaffinated in xylene and rehydrated in graded alcohol. To increase the immunoreactivity, microwave antigen retrieval was performed in citrate buffer (pH 6.0), and left to cool down at room temperature for 30 min. The sections were then incubated in hydrogen peroxide for 10 min, followed by incubation, in bovine serum albumin for 10 min. Subsequently, anti-Beclin-1 antibody was added to the