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Low expression of B-Cell-Associated protein 31 is associated with unfavorable prognosis in human colorectal cancer

Chong Ma[a,1], Ri-Ming Jin[b,1], Ke-Ji Chen[b,1], Tao Hao[a], Bao-Song Li[a], Da-Hua Zhao[c], Hong Jiang[a,*]

[a] Department of Colorectal and General Surgery, Binzhou Medical University Hospital, Binzhou, 256603, China
[b] The First Department of Liver Surgery, Eastern Hepatobiliary Surgery Hospital, Second Military Medical University, Shanghai, 200438, China
[c] Department of Pathology, Binzhou Medical University Hospital, Binzhou, 256603, China

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ABSTRACT

Colorectal cancer (CRC) is one of the most common cancers worldwide. B cell-associated protein 31 (BAP31) was shown to participate in the apoptosis, and to be an immunotherapy target and a, prognostic factor for cancer, but its role in CRC has not been elucidated. In this study, we examined the expression of BAP31 in CRC to evaluate its prognostic values. We investigated the BAP31 expression level in 142 tissues (108 CRC and 17 paired human adjacent normal mucosa, and 17 liver metastatic CRC tissues) from 108 patients, using tissue microarray-based immunohistochemistry. We further investigated the association between BAP31 expression and overall survival (OS) and disease-free survival (DFS) in 77 CRC patients using Kaplan-Meier analysis. Univariate and multivariate Cox regression analyses were applied to evaluate the potential prognostic value of BAP31 in CRC patients. BAP31 expression level was significantly increased in CRC tissues ($p = 0.0014$) and liver metastatic CRC tissues ($p < 0.0001$) compared with corresponding adjacent normal mucosa. BAP31 expression was also significantly increased in liver metastatic CRC tissues compared with corresponding primary CRC tissues ($p = 0.0116$). Kaplan-Meier analyses showed that CRC patients with low BAP31 expression had significantly lower survival rate ($p = 0.001$) and lower disease-free survival rate ($P = 0.009$). Furthermore, multivariate Cox analysis showed that BAP31 was an independent prognostic factor for OS (hazard ratio = 0.410, 95% confidence interval = 0.195–0.862, $p = 0.019$).

Conclusions: Our study demonstrated that BAP31 is a potential prognostic marker for CRC patients after surgery.

1. Introduction

Colorectal cancer (CRC) is one of the most common cancers worldwide and ranks fifth for both caner incidence (376.3 per 100,000) and mortality (191.0 per 100,000) in China [1].

Improvements have been made in preoperative staging, optimized surgical procedures, multidisciplinary treatment [2], and postoperative treatments including adjuvant chemotherapy (5-FU/LV, FOLFOX et al.) [3,4] and targeted therapy (bevacizumab, cetuximab, panitumumab) [5–7]. However, despite these, postoperative complications and recurrence persist, leading to a poor prognosis [8].

Therefore, there is a clear need to discover effective biomarkers to identify patients likely to have poor postoperative outcomes with the aim of guiding surgery and adjuvant treatment according to individual risk.

BAP31 is a shuttle protein acting between ER and Golgi apparatus which binds Bcl-2, caspase-1 and caspase-8 [9]. Several reports have shown that it is associated with human cancer. Specifically, BAP31 is a promising target for the immunotherapy of malignant melanomas [10], while low BAP31 expression is linked with poor prognosis of human primary hepatocellular carcinoma (HCC) [11]. Moreover BAP31 participate in calnexin-dependent regulation of tunicamycin-induced apoptosis in breast carcinoma MCF-7 cells [12]. However, the expression level and prognostic value of BAP31 in CRC have not yet been reported. The present study evaluated BAP31 protein expression levels using immunohistochemistry, and investigated its association with clinicopathological factors and the prognosis of postoperative CRC patients.

Abbreviations: CRC, colorectal cancer; BAP31, B-Cell-Associated protein 31; OS, overall survival; DFS, disease free survival; FFPE, formalin-fixed paraffin-embedded; HE, hematoxylin and eosin; IHC, immunohistochemistry; HCC, hepatocellular carcinomas

* Corresponding author at: Department of Colorectal and General Surgery, Binzhou Medical University Hospital, No. 522, Huanghe Sanlu, Bincheng District, Binzhou City, Shandong, 256603, China.
E-mail address: bzbh519@163.com (H. Jiang).
[1] These authors contributed equally to this study.

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2. Materials and methods

2.1. Patient tissue samples

Formalin-fixed paraffin-embedded (FFPE) specimens were collected from 108 CRC patients treated at the Binzhou Medical University Hospital between April 2009 and March 2011. Among them 17 CRC tumors with paired adjacent normal mucosa and liver metastatic colorectal cancer tissues were obtained. Additionally, 77 cases with overall survival (OS) times and 72 cases with disease-free survival (DFS) times were available for analysis.

The diagnosis CRC was based on clinical manifestation, pathological and serological examinations. The follow-up time was calculated from the date of surgery to the date of death, or the last known follow-up. All patients enrolled in this study provided written informed consent, and the study was approved by the Ethics Committee of Binzhou Medical University Hospital. No patient had received radiotherapy, chemotherapy, hormone therapy, or other related anti-tumor therapies before surgery.

OS was defined as the length of time between surgery and death or the last follow-up examination. DFS was calculated from the date of tumor resection until the detection of tumor recurrence, metastasis, or death at the last observation. Follow-up was performed every 3 months in the first year after surgery, every 3–6 months for the next 4 years, and every 6–12 months thereafter; follow-up data were summarized at the end of April 2016.

Follow-up of all patients was carried out in Binzhou Medical University Hospital, and included a tumor marker check every 3 months and diagnostic imaging check at least every 6 months, which was also based on the surveillance suggested in the guidelines. In cases of suspected recurrence, MRI or CT was added to the diagnostic imaging [8].

2.2. Tissue microarrays and immunohistochemistry (IHC)

One hundred and forty-two specimens (108 primary CRC specimens and 17 paired adjacent normal mucosa tissues, and 17 paired liver metastatic CRC specimens) were collected randomly. Tissue microarrays were constructed from the representative core of each specimen as reported previously [13]. In brief, Hematoxylin and eosin-stained slides were made from each tissue specimen, and reviewed by experienced pathologists; representative cores were pre-marked in the paraffin blocks. Tissue cylinders with a diameter of 1.5 mm were punched from the marked areas of each block and incorporated into a recipient paraffin block. Sections of 4 μm-thick were placed on slides coated with 3-aminopropyltriethoxysilane.

Paraffin sections were deparaffinized in xylene and rehydrated through decreasing concentrations of ethanol (100%, 95%, and 85% for 5 min each). Antigens were unmasked by microwave irradiation for 5 min in pH 6.0 citric buffer and cooled at room temperature for 60 min. Endogenous peroxidase activity was blocked by incubation of the slides in 3% H_2O_2/phosphate-buffered saline, and non-specific binding sites were blocked with goat serum [14]. The primary antibody was a rabbit polyclonal antibody against BAP31 (ab37120; abcam, USA; 1:4000 dilution, cytoplasmic staining). An EnVision Detection kit (GK500705: Gene Tech, Shanghai, China) was used to visualize tissue antigens. Tissue sections were counterstained with hematoxylin for 5 min. Negative control slides omitting primary antibody were created for all assays. The image system comprised a Leica CCD camera DFC420 connected to a Leica DM IRE2 microscope (Leica Microsystems Imaging Solutions Ltd, Cambridge, UK). Photographs of representative fields were captured under high-power magnification (×200) using Leica QWin Plus v3 software. For semiquantitative evaluation of BAP31, the scoring was as follows: the staining intensity was first scored (0, negative; 1, weak; 2, moderate; 3, high) and then the percentage of positive cells was scored (0, 0% positive cells; 1, 1–10% positive cells; 2, 11–50% positive cells; 3, > 50% positive cells). The final score of each sample was obtained by multiplying the two scores together [15].

2.3. Statistical analysis

Kaplan-Meier analyses, the chi-square test, correlations between variables, univariate survival analysis and multiple Cox proportional hazards regression were performed using the SPSS statistical software package (SPSS Standard version 13.0; SPSS, Chicago, IL, USA). For 2×2 tables, if minimum expected count > 5, and N of valid cases > 40, we adopted Person's chi-square test. For 2×2 tables, if 1 < minimum expected count < 5, and N of valid cases > 40, we adopted Continuity Corrected chi-square test.

We selected the optimum cutoff score for the expression of BAP31 using X-tile plots based on the association with OS and DFS. X-tile plots provide a single and intuitive method to assess the association between variables and survival. The X-tile program can automatically select the optimum data cut point according to the highest $\chi 2$ value (minimum p value) defined by Kaplan–Meier survival analysis and log-rank test [16]. We generated X-tile plots using X-tile software version 3.6.1 (Yale University School of Medicine, New Haven, CT, USA). A significant difference was considered if the P value from a two-tailed test was less than 0.05.

3. Results

3.1. Expression levels of BAP31 in paired adjacent normal mucosa, CRC tumor tissues and liver metastatic CRC tissues

As shown in Fig. 1, BAP31 was negatively expressed or expressed at low levels in adjacent normal mucosa (Fig. 1A), and highly expressed (Fig. 1B) in CRC tumor tissues and liver metastatic CRC tissues (Fig. 1C). Significant differences were observed between 17 paired adjacent normal mucosa and CRC tumor tissues ($p = 0.0014$), and between 17 paired adjacent normal mucosa and liver metastatic CRC tissues ($p < 0.0001$) using the paired *t*-test (Fig. 1D). Notably, BAP31 expression was slightly higher in liver metastatic CRC tissues compared with paired primary CRC tissues

3.2. Kaplan–Meier analyses according to cut-off point obtained from X-tile analyses

X-tile analysis showed that the optimal cut-off point for OS and DFS was 4 (Fig. 2). Thus, if IHC score was equal or smaller than 4, the patient was categorized into the low BAP31 expression group, while those with scores above 4 were categorized into the high BAP31 expression group. As a result, 21 patients were placed in the high BAP31 expression group, and 56 patients in the low BAP31 expression group. Kaplan–Meier analyses clearly showed that the OS and DFS were relatively poor for CRC patients with low BAP31 expression in their primary tumor tissues compared with those patients with high BAP31 expression ($p = 0.001$ for OS, $p = 0.009$ for DFS) (Fig. 3).

3.3. Association of BAP31 expression with clinicopathological features of CRC patients

Based on the cut-off point determined by X-tile analyses, we then investigated the association between BAP31 expression and clinicopathological factors in 108 CRC patients. Chi-square analysis showed that BAP31 expression was not associated with any clinicopathological factors except N stage ($p = 0.026$) in CRC patients (Table 1). Chi-square analysis also showed that liver metastasis was significantly associated with N stage ($p = 0.35$), M stage ($p < 0.0001$), TNM stage ($p < 0.0001$), serum CEA ($p = 0.17$), serum CA19-9 ($p = 0.005$) (Table 2).