Original Paper

Lower Bmi-1 Expression May Predict Longer Survival of Colon Cancer Patients

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Key Words

Bmi-1 • Colon cancer • Clinical significance • Survival

Abstract

Background: This study aimed to investigate the Bmi-1 expression and the clinical significance in colon cancer (CC). **Patients and Methods:** Bmi-1 expression in tumor tissue and the corresponding normal tissue was detected using immunohistological staining. The correlations between Bmi-1 expression and clinicopathological characteristics and the overall survival (OS) time were analyzed. **Results:** The median H-scores of Bmi-1 in CC tissues and the corresponding tissues were 80.0 (0–270) and 5.0 (0–90), with no statistically significant difference ($Z = -13.7$, $P < 0.001$). Bmi-1 expression in CC tissues was not statistically correlated with any characteristics. The median OS times for CC patients with high or low Bmi-1 expression were 53.7 months and 44.9 months, respectively, with no statistically significant difference ($P = 0.123$). The survival rates of patients with low Bmi-1 expression were higher than those of patients with high Bmi-1 expression but the differences were not statistically significant. **Conclusion:** Bmi-1 expression in CC tissue is significantly higher than that in corresponding normal tissue. While there may be a trend towards improved survival, this is not statistically significant.

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Introduction

Colon cancer (CC) is the fourth common malignancy worldwide [1]. In China, both the incidence and mobility rate are increasing recently [2].

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Bmi-1 is a member of the polycomb group which functions as a transcriptional repressor [3, 4]. *Bmi-1* gene is associated with tumor invasion and metastasis [5]. Several lines of evidence suggest that Bmi-1 blocks cell senescence and proliferation [6, 7]. Aberrant expression of Bmi-1 has been detected in colorectal carcinoma, breast carcinoma, prostate cancer, liver carcinoma, medulloblastoma, lymphoma, acute myeloid leukemia, head and neck squamous cell carcinoma, non-small cell lung cancer and glioblastoma [5, 6, 8-13]. Bmi-1 has been identified as a predictor of the response to therapy and poor survival of patients with various tumors [14-16].

Bmi-1 has been detected in colorectal cancer tissue [17-20] but with smaller sample sizes. In this study, we investigated the expression of Bmi-1 in CC using a larger sample size of tumor tissue and analyzed the correlation between Bmi-1 expression and clinical significance and outcome. We aimed to confirm the potential role of Bmi-1 as a biomarker for the prognosis of CC.

Patients and Methods

Patients and samples

This study was approved by the Ethics Committee of The Third Affiliated Hospital of Soochow University (also named The First People's Hospital of Changzhou). Human CC high density tissue microarray obtained from Shanghai Outdo Biotech Co. Ltd. contained 267 primary tumor specimens and the corresponding tissue with follow-up information included. The patients' baseline clinicopathological characteristics are listed in Table 1. Some of the patient's information was not available and thus were secluded from the statistical analysis.

Immunohistochemistry (IHC)

IHC analysis of Bmi-1 was performed using a standard streptavidin-biotin-peroxidase complex method described in our previous article [21]. Two pathologists with no knowledge of the patients' information examined the stained sections independently. The Bmi-1 immunostaining densities were assessed according to the H-score method described [22]. $H\text{-score} = (\% \text{ of tumor cells unstained} \times 0) + (\% \text{ of tumor cells stained weak} \times 1) + (\% \text{ of tumor cells stained moderate} \times 2) + (\% \text{ of tumor cells stained strong} \times 3)$. The H-scores ranged from 0 (100% negative tumor cells) to 300 (100% strong staining tumor cells). Results from the two pathologists were averaged and used for statistical analysis.

Statistical analysis

All data were analyzed with SPSS statistical software (SPSS Standard version 13.0, SPSS Inc.). The Bmi-1 expression between tumor and the corresponding tissues were compared with the Wilcoxon test. The association between Bmi-1 expression and the clinicopathological characteristics was assessed with the chi-square test. For univariate survival analysis, all CC patients were analyzed by Kaplan-Meier analysis. The log-rank test was used for comparing different survival curves. A *P* value less than 0.05 was considered statistically significant. Data were expressed as mean $\pm$ standard error.

Results

Patient baseline characteristics

Table 1 summarizes the patients' available clinicopathological characteristics. There were 127 male and 140 female cases. There were 17 cases with stage I, 132 with stage II, 107 with stage III and 9 with stage IV.

Correlation between Bmi-1 expression and patient's clinicopathological characteristics

Immunoreactivity for Bmi-1 protein was observed as brown, granular staining on the cell nuclei of tumor cells (Fig. 1). The cut-off value of Bmi-1 expression was 135. The median H-scores of Bmi-1 in CC tissues and the corresponding tissues were 80.0 (0-270) and 5.0 (0-

90), with no statistically significant difference ($Z = -13.7$, $P < 0.001$).

Univariate analysis revealed that there was no statistically significant difference between Bmi-1 expression level and any clinicopathological characteristics (including age, gender, pathological differentiation degree, tumor size, lymph node involvement status, distant metastasis status and TNM stage, all $P > 0.05$). See Table 1.

Correlation between Bmi-1 expression and the overall survival (OS) time and survival rates

Based on the Kaplan-Meier survival analysis, cumulative survival curves were calculated, and differences in survival time were assessed with the log-rank test. The median OS times for CC patients with high or low Bmi-1 expression were 53.7 months and 44.9 months, respectively ($P = 0.123$). See Figure 2.

Table 2 summarizes the 1-year to 5-year of CC patients. For patients with high Bim-1 expression ($n = 74$), the 1-year to 5-year survival rates were 75.7%, 67.6%, 59.5%, 50.3% and 46.3%, respectively. For patients with low Bim-1 expression ($n = 193$), the 1-year to 5-year survival rates were 81.3%, 72.5%, 66.8%, 61.1% and 56.5%, respectively. We found that the survival rates of patients with low Bmi-1 expression were higher than that of patients with high Bmi-1 expression but none of the differences of various years were statistically significant ($P > 0.05$). See Table 2.

Table 1. Correlation between patient clinicopathological characteristics and Bmi-1 expression. #Tumor size was defined as the largest diameter of the tumor mass

Characteristics	Bmi-1 expression				χ^2	P
	Lower No.	%	Higher No.	%		
Age					4.476	0.107
≤ 60	42	82.4	9	17.6		
> 60 and ≤ 70	75	72.8	28	27.2		
> 70	73	66.4	37	33.6		
Gender					0.588	0.443
Female	89	70.1	38	29.9		
Male	104	74.3	36	25.7		
Pathological differentiation					0.572	0.751
I	13	68.4	6	31.6		
II	122	71.3	49	28.7		
III	58	75.3	19	24.7		
Tumor size #					1.195	0.274
≤ 5cm	113	74.3	39	25.7		
> 5cm	75	68.2	35	31.8		
Lymph node involvement					0.079	0.779
No	106	71.1	43	28.9		
Yes	80	72.7	30	27.3		
Distant metastasis					1.295	0.255
No	184	71.6	73	28.4		
Yes	8	88.9	1	11.1		
TNM staging					1.724	0.632
I	11	64.7	6	35.3		
II	95	72.0	37	28.0		
III	77	72.0	30	28.0		
IV	8	88.9	1	11.1

Fig. 1. Immunohistochemical analysis of Bmi-1 showing representative images negative (A) staining in the corresponding tissues and weak (B), moderate (C) and strong (D) staining and in the tumor cells of colon cancer. Bmi-1 protein is observed as brown, granular staining on the cell nuclei of tumor cells.

Table 2. Association between Bim-1 expression and survival rates of patients with colon cancer

Bmi-1 expression (n)	1 year (%)	2 year (%)	3 year (%)	4 year (%)	5 year (%)
Higher (74)	75.7	67.6	59.5	50.3	46.3
Lower (193)	81.3	72.5	66.8	61.1	56.5

Fig. 2. Survival curves of colon cancer patients with high and low Bmi-1 expression levels. There is no statistically significant difference between the overall survival times of the two groups of patients ($P = 0.123$).

Discussion

In the present study, we found that the OS of patients with low Bmi-1 expression tended to be longer, however not significant.

Other investigators also find that low Bmi-1 expression had a better survival outcome [20, 23]. And Bmi-1 immunoreactivity emerged as an independent prognostic factor [19]. In pancreatic cancer [4], patients with low Bmi-1 expression had a better survival outcome. We have meta-analyzed the association between Bmi-1 expression and the clinicopathological characteristics and prognosis of patients with various malignancies [24]. The results suggest that high Bmi-1 expression predicts poorer clinical outcome.

However, Espersen et al. observed Bmi-1 expression in primary tumors of stage II colon cancer patients could not predict relapse or OS of the patients, thus having a limited prognostic value in stage II colon cancer patients [25]. Furthermore, some other studies suggest opposite conclusions to ours. In breast cancer, patients with high levels of Bmi-1 have a more favorable outcome than patients with low expression of Bmi-1 [26]. And we recently found that high levels of Bmi-1 in gastric cancer patients are significantly associated with better OS ($P = 0.02$) [21].

The differences among the results of various studies might be explained by the complex function of polycomb protein Bmi-1. Bmi-1 functions as a double-edged sword. It has been reported that leukemic stem cells lacking Bmi-1 do not proliferate [27, 28]. Bmi-1 protein might be involved in human colorectal carcinogenesis [17]. Bmi-1 is required for colon cancer proliferation *in vitro* and *in vivo* [29]. Downregulation of Bmi-1 inhibits cell proliferation and cycle progression and promotes apoptosis of tumor cells of colorectal cancer [30].

Chemoresistance can be facilitated via the upregulation of Bmi-1 [31]. Reducing Bmi-1 expression can inhibit the proliferation of tumor cells and inverse chemoresistance [32]. Meanwhile, tumor cells with high Bmi-1 expression proliferate faster and hence are more sensitive to chemotherapy, leading to longer survival.

Furthermore, our sample size is larger than other studies, although the size alone does not explain the difference of results. Majority of our patient population has stage II and III disease; the omission of stage I and IV patients may alter the results. Besides, some information is unavailable so it is not certain how treatment modalities influence survival. Treatment effect on outcome and clinical follow-up for recurrences will further strengthen the findings. This may also be a reason why there is no significant difference in the OS times between the two groups of patients.

Conclusively, while there may be a trend towards improved survival, this is not statistically significant.

Acknowledgements

This work was supported by grants from National Natural Science Foundation of China (No. 31570877 and 31570908), Jiangsu Engineering Research Center for Tumor Immunotherapy (BM2014404), and Society development plans, Department of Science and Technology Changzhou (CJ20159018).

Disclosure Statement

The authors declare that they have no conflict of interest.

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