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Galectin-3 expression in colorectal cancer and its correlation with clinical pathological characteristics and prognosis

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Abstract: Objective: To explore the expression levels of galectin-3 in colorectal cancer and the association between galectin-3 and its clinical pathological parameters, as well as the prognosis of colorectal cancer patients. Methods: An immunohistochemistry assay was used to test the expression levels of galectin-3 in cancer tissues of 61 colorectal cancer cases and in normal intestinal tissues adjacent to the cancer tissues of 23 cases. The associations between protein expression levels of galectin-3 and the clinicopathological features, such as age, sex, pathology type, lymphatic metastasis, and prognosis were also analyzed. Results: The positive rate of galectin-3 in cancer tissues was significantly higher than that of cancer-adjacent tissues: 62.5% (38/61) versus 13.0% (3/23) ($P<0.05$), respectively. Correlation was found between the protein expression of galectin-3 and the tumor size ($P<0.05$), as well as between the tumor differentiation ($P<0.05$) and Duke staging ($P<0.05$). The median progression-free survival times of patients with galectin-3 positive and negative expression were 19.2 and 35.1 months, respectively, with significant statistical difference ($P<0.05$). Conclusion: Galectin-3 expression was correlated with the genesis and development of colorectal cancer and which could be used a biological marker for the prognosis of colorectal cancer patients.

Keywords: Colorectal cancer; Galectin-3; Prognosis; Immunohistochemistry

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1 Introduction

With the advances in molecular biology, colorectal cancer is now considered by most investigators to be a pathological process involving multiple genes and steps [1,2]. Activation of oncogenes and inactivation of anti-oncogenes play important roles during this process [2-4]. Galectin-3 belongs to the galectin family, which is involved in multiple biological behaviors, such as cell proliferation, apoptosis, and adhesion via combination with different proteins [5]. Elevated expression of galectin-3 was observed in tissues of multiple solid malignant tumors, whereas low or no expression was observed in normal tissues [6-8]. In the present study, an immunohistochemical assay was performed to measure the expression levels of galectin-3 in colorectal cancer tissues, as well as in tissues adjacent to the cancerous ones in order to explore the correlation between galectin-3 expression and clinical pathological features, as well as prognosis.

2 Material and methods

2.1 Clinical samples

Colorectal cancer patients who were surgically treated in our general surgery department from March 2012 to January 2015 were selected as study objects. The inclusion criteria was described as follows: colorectal cancer was validated by pathology, clinical medical data were complete for the patients with complete follow-up data, and histopathological sample testing was approved in written form by the patient or his/her family. However, patients who met the following criteria were excluded from the

study: unclear histopathological diagnosis, neoadjuvant chemoradiotherapy, incomplete follow-up data, and complicated history of other malignant tumors. Cancer tissue samples from all the 61 subjects and adjacent tissue samples from 23 patients were included in the study. The average age of the 61 patients was 65.1±15.65 years. For the included 61 patients, there were 33 male and 28 female with 27 colon cancer cases and 34 rectal cancer cases. 36 patients were diagnosed at A/B staging and 25 patients at C/D staging according to the Duke staging system.

Ethical approval: The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the authors' institutional review board or equivalent committee.

2.2 Instruments and reagents

A paraffin slicing machine (Leica, Germany), microscope (Olympus, Japan), electrothermostat (Shezhen Ruitai Co., Ltd., China), microwave oven, conventional centrifuge (Beijing BaiYangMedical Devices Co., Ltd.),purified water system (Millipore, Germany),mouse anti-human galectin-3 monoclonal antibody (Santa Cruz Biotechnology, USA), immunohistochemical staining kit SP-9000 (Beijing Zhongshan Biotechnology Co.,Ltd., China), and DAB Displaying Kit (Beijing Zhongshan Biotechnology Co., Ltd., China) were used in this study.

2.3 Immunohistochemical assay

Paraffin wax samples of cancer tissues and adjacent tissues were incised into paraffin sections with a diameter of 4 μm. Histological sections were first soaked into dimethylbenzene for 10min, followed by dehydrated alcohol for 5 min, and then in succession in the following alcohol solutions for 5 min each for dewaxing with 95%, 80%, and 79% alcohol. These slices were soaked in 3% H_2O_2 (30% H_2O_2:methanol=1:9) and then incubated in the dark for 15min. To inactivate endogenous peroxidase, 0.01% mol/L sodium citrate was added to the sections. The sections were then placed in a water bath heated at 95°C for 15 min. A drop of 100μl of normal serum containing 10% diluted TBST was added to the tissues. These tissues were incubated over night at 4°C. A 1:100 secondary antibody was added with HRP marking for incubation under room temperature for 1 h. Coloration with the coloring reagent was performed by washing the samples with dH_2O twice for 5 min to 10 min. Afterward, the sections were sealed. The sections were then dried prior to observation under a microscope. The samples were evaluated by cell staining with the following scores: brown, 3 points; pale brown,2 points; light brown,1 point; and no coloration, 0 points. The quantity of stained cells was evaluated with the following scores: quantity of stained cells in one visual field of>75%, 4 points; 51% to 75%, 3points; 11% to 50%, 2 points; <10%, 1 point; and negative, 0 points. The final result is achieved by multiplying the scores from the two categories: 0 to 3 points, negative expression (−); 4 to 6 points, weakly positive expression (+); 7 to 9 points, fairly strong positive expression (++); and 9 to 12 points, strong positive expression (+++).

2.4 Follow up

Periodic follow up by phone or outpatient service was performed after therapy. Hospitalized treatment was performed for patients requiring adjuvant chemotherapy in accordance with the NCCN guideline. The disease progression and time of death of patients (in case of mortality) were recorded.

2.5 Statistical analysis

For statistical analysis, $\overline{x} \pm S$ was used to represent the measurement data for each patient, whereas t-test was used for group comparison. The ratio was used to represent the enumeration data and compared by Chi-square test. The survival data was represent by median. The log-rank method of Kaplan–Meier survival curve was used to compared the survival difference of the two groups. Statistical significance was determined for bilateral $P<0.05$. SPSS16.0 software was used for all statistical analyses in this study.

3 Results

3.1 Expression of galectin-3

In this study, galectin-3 was primarily expressed in the cytoplasm and cell membrane with yellow-brown gran-

ules. The positive rates of galectin-3 in cancer tissues and adjacent normal tissues were 62.5% (38/61) and 13.0% (3/23), respectively. Galectin-3 positive expression rate was significantly higher in cancer tissues than that in adjacent normal tissues (P<0.05) , Figure 1.

3.2 Correlation between Galectin-3 expression and patients clinicopathological features

Correlation was found between the protein expression of galectin-3 and the tumor size (P<0.05), tumor differenti-

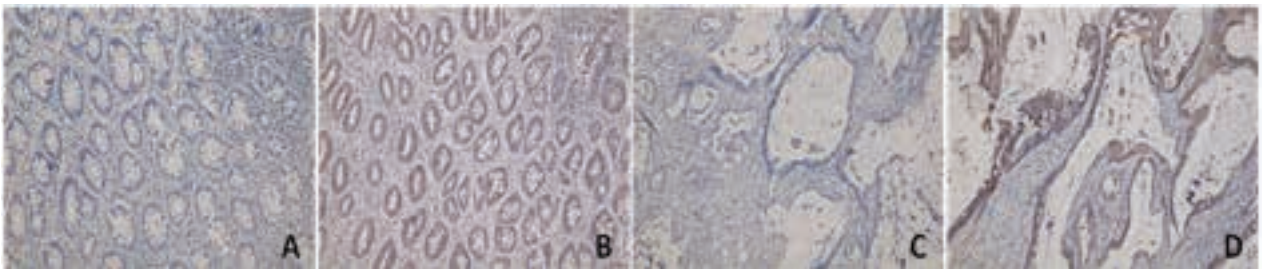

Figure 1: Galectin-3 expression in colorectal cancer tissue and normal intestinal tissues by immunohistochemistry
(A: galectin-3 negative expression in normal intestinal tissues, ×200; B: galectin-3 positive expression in normal intestinal tissues, ×200; C: galectin-3 negative expression in colorectal cancer tissues, ×400; D: galectin-3 positive expression in colorectal cancer tissues, ×400)

Table 1: The association between Galectin-3 protein expression and clinical pathological characteristics

Characteristics	n=61	Galectin-3 expression		Chi-square	P
		Positive (n=38)	Negative (n=23)		
Age(years)				0.0	0.91
≤50	17	11	6		
>50	44	27	17		
Gender				0.14	0.71
Male	33	22	11		
Female	28	16	12		
Tumor location				0.39	0.53
Colon	27	18	9		
Rectal	34	20	14		
Pathology grading				6.99	**0.01**
Well-differentiated	13	4	9		
Poor-differentiated	48	34	14		
Tumor diameter(cm)				5.19	**0.02**
>5	30	23	7		
≤5	31	15	16		
Duke stage				5.65	**0.02**
A/B	36	18	18		
C/D	25	20	5		
Tumor gross type				1.08	0.58
Mass forming	25	15	10		
Infiltrative	27	16	11		
Ulcerative	9	7	2		

ation (P<0.05) and Duke staging (P<0.05). But it was not correlated with patient's gender(P>0.05), age (P>0.05), tumor location (P>0.05), and gross type (P>0.05), Table 1.

3.3 Galectin-3 expression and prognosis

The median progression-free survival time for patients with positive and negative galectin-3 expression were 19.2 and 35.1 months, respectively. The risk of disease progression for patients with positive galectin-3 expression was significantly higher than that for patients with negative galectin-3 expression (HR=2.10, 95%CI:1.05 to 4.17, P<0.05), Figure 2.

4 Discussion

In 2012, clinical epidemiology studies reported that about 140,000 patients were diagnosed with colorectal cancer in North America; up to 50,000 patients died from colorectal cancer in the same year [9]. No authoritative and accurate epidemiological data are available for colorectal cancer in China [10]. On the basis of the incidence of colorectal cancer from several provinces, this malignancy ranks behind the incidence of lung cancer, breast cancer, and gastric cancer [11-14]. The exact cause of colorectal cancer remains unclear. Most studies regarded colorectal cancer to be associated with a family history of tumors, adenoma of the colon, high-fat and high-protein diets, and limited amounts of exercise [15-17].

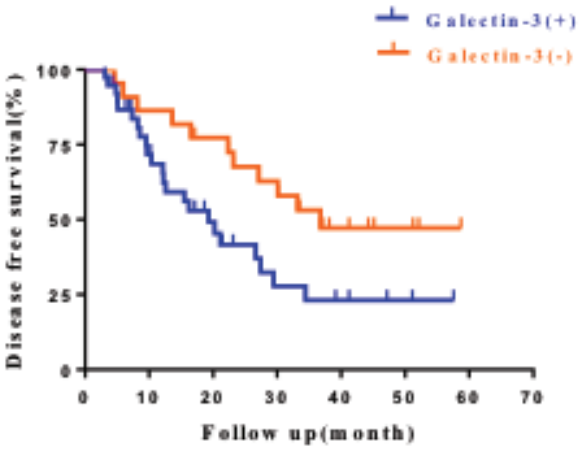

Figure 2: The disease survival curve comparison between galectin-3 positive and negative patients

Recent studies showed that the inactivation of certain anti-oncogenes and the activation of oncogenes play important roles in the genesis and progression of colorectal cancer [18]. Relevant protein factors are particularly important in the activation of oncogenes or the significant increase in their expression levels. Galectin-3 is one of the members of galectin protein family. These proteins are involved in multiple biological behavior, such as cell proliferation, apoptosis, and adhesion. In humans, the galectin-3 gene is located at 14q21- 22 and is composed of 6 exons and 5 introns. Galectin-3 protein is produced in the nucleus and localized mainly in the cytoplasm at the cell surface and outside the cell, exerting multiple effects via interaction with corresponding ligands. Clinical studies demonstrated that galectin-3 is highly expressed in tissues of multiple solid tumors, including esophageal cancer, gastric cancer, lung cancer, and liver cancer. Moreover, galectin-3 expression is correlated with a patient's prognosis [19-22]. Wan et al [23] employed an immunohistochemical method to test the expression level of galectin-3 in tissues of gastric cancer and determine its correlation with the pathological features and prognosis of patients. Studies showed that the positive expression rate of galectin-3 in tissues of gastric cancer is relatively high and correlated with invasive depth and lymphatic metastasis. Li et al [24] reported that galectin-3 expression is significantly higher in colorectal cancer tissues than that of normal intestinal tissue. These reports conclude that galectin-3 plays an important role in the genesis and progression of colorectal carcinoma.

In the present study, we found that the positive expression rate of galectin-3 in colorectal cancer tissues was 62.5% (38 / 61), whereas the positive expression rate of galectin-3 in normal tumor-adjacent intestinal epithelial tissues was 13.0% (3 / 23). The positive rate of cancer tissues was significantly higher than that of normal tumor-adjacent tissues. This finding is consistent with the report of Li et al [24], suggesting that galectin-3 may be involved in the genesis and progression of colon cancer. We also found that tumor size was relatively large with low differentiation extent and late clinical staging in patients with positive expression. These parameters are indicators of relatively poor prognosis. Analysis of the association between the expression level of galectin-3 and the survival rates indicated that the median progression-free survival time of patients with positive galectin-3 expression was significantly shorter than that of patients with negative expression. This result indicated that the positive expression of galectin-3 was associated with more malignant biological behavior of colorectal cancer and can be used as a predictor of poor prognosis for patients. The associ-

ation between galectin-3 expression and clinical features, as well as the prognosis for patients, is evident, but the molecular mechanism on how galectin-3 controls genesis, progression, invasion, and metastasis of colorectal cancer remains unclear [25, 26]. Therefore, immunoblotting and co-immunoprecipitation assays should be further performed to explore the underlying molecular mechanisms controlling the malignant biological behavior of colorectal cancers.

Conflict of interest statement: Authors state no conflict of interest

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