EXPRESSION OF GALECTIN-3 IN GASTRIC CARCINOMA AND ITS SIGNIFICANCE

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CLC number: R735.2 Document code: A Article ID: 1000-9604(2004)03-0176-06

ABSTRACT

Objective: To study the expression of Galectin-3 in gastric cancerous tissues and the relationship between Galectin-3 expression and biological behave of gastric carcinoma. Methods: S-P immunohistochemistry method was used to examine Galectin-3 expression in 64 primary gastric cancerous tissues and 10 normal gastric mucosa as control. The expression of Galectin-3 was examined by HPIAS- 2000 image analysis software. Results: Positive Gaiectin-3 expression was observed in 85.9% of the gastric cancer cases. In gastric cancerous tissues, about 46% of cases showed strong nuclear immunoreactivity. The degree of enhancement of immunoreactivity was different in various histopathological subtypes in cancerous tissues. The readings of average absorbency of Galectin-3 in well, moderate and poorly differentiated adenocarcinoma tissues were 0.1713±0.02147, 0.1873±0.0313 and 0.2538±0.1216 respectively. A stronger expression of Galectin-3 in cancerous tissues was observed. When Galectin-3 expression in gastric cancerous tissues was compared with that in normal gastric tissues, there was a significant difference (P<0.001). Conclusion: A significantly stronger expression of Galectin-3 in gastric cancerous tissues was observed. Galectin-3 might be a useful tumor marker for gastric carcinomas with respects to tumor metastasis, proliferation, cancer progression, and tumor cell adhesion.

Key words: Galectin-3; Gastric carcinoma; Immunoreactivity

Galectin is a large family of β-galactoside-binding lectins with wide tissue distribution from lower invertebrates to higher vertebrates[1]. Among them, Galectin-3 a M(r) 31000 member of the beta-galactoside-binding protein family, is a multifunctional protein implicated in a variety of biological functions, including tumor cell adhesion, proliferation, differentiation, angiogenesis, apoptosis, cancer progression, and metastasis[2]. The implication of Galectin-3 during malignancy progression has been suggested in several cancers, including colon, prostate, thyroid, and breast cancer, however, scarce data are available in gastric cancer[3].

In this study, we examined the expression of Galectin-3 in 64 gastric carcinomas by immunohistochemistry methods of S-P to explore whether it is related to the malignant progression and the metastatic lymph nodes.

MATERIALS AND METHODS

Specimens

All paraffin-embedded tissue sections were obtained from the Renmin Hospital of Wuhan University from October 1999 to May 2003. There were intact pathological diagnosis materials. Sixty-four patients (46 males, 18 females, aged between 25 to 78 y, median 55.7 y) were as follows: 12 cases of well differentiated adenocarcinoma, 21 cases of moderately differentiated adenocarcinoma, 31 cases of poorly differentiated adenocarcinoma. 42

Received date: May 28, 2003; **Accepted date:** Jul. 26, 2004
Foundation item: This work was supported by a grant from Hubei Province Education Committee (No. 2003X 124).
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cases with metastatic lymph nodes, 22 cases with no metastatic lymph nodes; Normal control group of 10 normal gastric tissues were organized. Each paraffin-embedded tissue sections were cut into 2 slices in succession of 4 μm thick, one was stained by hernatoxylin-eosin (H&E) to reexamine the diagnose, another was performed by immunohistochemical staining.

Reagents

The monoclonal mouse Anti-Galectin-3 was purchased from Beijing Zhongshan Biological Technology Ltd (Beijing, China). S-P immunohistochemical kit and DAB kit were purchased from Fujian Maixin Biological Technology Ltd (Fujian China).

Immunohistochemistry

Immunohistochemical staining was performed using S-P method in large batches over a period of several days. Paraffin-embedded samples were cut. Tissue sections were dewaxed in xylene for 30 min, rehydrated through graded alcohol to PBS (pH 7.4), then immersed in 3% hydrogen peroxide at room temperature for 10 min to quench endogenous peroxidase activity. After washed with PBS, the section were subjected to antigen retrieval in boiling sodium citrate buffer (0.01 mmol/L pH 6.0) for 10 min in a microwave oven set at 95~100°C. After cooled at room temperature, and washed with PBS and distilled water sequentially, 100 μl of diluted anti-Galectin-3 mouse antibody from Zhongshan Biological Corp (Beijing, China) (1:100) was applied for each section. After washed with PBS, each of the sections was incubated at 37°C for 10 min with 100 μl of goat anti-mouse IgG from Maixin Biological Corp (Fujian, China). After washed with PBS again, the sections were subjected to sequential 3, 3-diaminobenzidine (DAB Kit, Maixin Biological Corp, Fujian, China) for immune complex visualization and then counterstained with hematoxylin for 30 seconds. All sections were counterstained with hematoxylin, and dehydrated through a graded alcohol series terminating with xylene. Formalin-fixed and paraffin-embeded sections of human thyroid papillary carcinoma with strong staining served as positive control whereas PBS in lieu of anti-Galeetin-3 as negative control. The Galectin-3 staining was independently reviewed by two immunohistochemistry experts. Microscopically, the slides with no staining in negative control and specific dark yellow staining of cytoplasm and nuclear membrane in positive control were eligible for further analysis.

Immunohistochemistry Assay for Galectin-3

A semi-quantitative evaluation was used to determine positively expressed cells by viewing 10 vision fields at × 400. All sections was analyzed by HPIAS 2000 image analyse software of Tongji Qianping Image Engineering Company, Wuhan China. Negative (-) indicated less than 10% stained cells, mild positive (+) showed 10%~30% stained cell, moderately positive (++) demonstrated 30%~60% stained cell, strong positive (+++) revealed over 60% stained cells. The later three grades were all regarded as positive. Staining was also categorized as cytoplasmic/membranous nuclear, or both.

Statistical Analysis

All statistical analysis was carried out with SPSS11.5 Software. The relationship between Galectin-3 expression and categorical variables was compared with x^2 test or fisher tow-sided exact test. Continuous variables were analyzed with t test and $P<0.05$ was considered as statistically significant.

RESULTS

Localization of Galectin-3 Protein

Immunohistochemical assay demonstrated that Galectin-3 mainly existed in cytoplasm, could also be detected in cell nucleus, cell surface or outside cell[4]. The staining was weak yellow, dark yellow and brown at a low power field and diffuse or granular staining at a high power field under microscopy.

Expression of Galectin-3 in Gastric Tissues

There were 55 positive staining specimens in 64 gastric cancerous tissues, total positive rate was 85.9%. The positive rate of nuclear staining of Galectin-3 in gastric cancerous tissue was 46%. A weak staining of Galectin-3 was observed in chronic gastritis tissue with a positive rate of 23.79% (Figure 1). The rates of positive staining of Galectin-3 in well, moderately and poorly differentiated gastric cancerous tissue were 62.91%, 69.83% and 63.26% respectively. The average absorbencies of well, moderately and poorly differentiated gastric cancerous tissue were 0.1713 ± 0.02147, 0.1873 ± 0.0313 and 0.2538 ± 0.1216 (Table 1). Poorly differentiated tumors stained more intensely than moderately differentiated tumors, moderately differentiated tumors stained more intensely than well