

Lectin Histochemical Study of Cell Surface Glycoconjugate in Gastric Carcinoma Using Helix Pomatia Agglutinin

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Abstract- Altered glycosylation of proteins in cancer cells is one of the main processes responsible for anaplasia, invasion and metastatic potential of neoplastic cells. Lectins are nonimmunogenetic compounds which specifically detect certain terminal sugars of glycoconjugates. The aim of the present study was to identify the N-acetylgalactosamine (GalNac) containing glycoconjugates in cancer cells in all grades of gastric carcinoma. Paraffin blocks belong to 30 patients of gastric carcinoma (10 cases from each grade) was collected from pathology file of Ali-Ebn-Abitaleb Hospital in Zahedan during 2005-2007. Prepared sections (5-7µm in thickness) were stained by Alcian Blue, hematoxylin and eosin (H&E) and helix pomatia agglutinin (HPA) conjugated lectin. Lectin diluted up to 10µg/ml in PBS (0.1M, pH=6.8). Lectin reactivity was visualized by 0.03% diaminobenzidine (DAB) solution. Sections were graded according to staining intensity to lectin (0-4+). Although there was some difference for lectin staining intensity between cancer cells in different grades of gastric carcinoma, statistical analysis showed that there was only a significant difference for cancer cells reactivity between histopathological grades of II and III. The pattern of reactivity to HPA lectin were also different from all histopathological grades. It seems that in cancer cells, the amount and distribution of GalNac containing glycoconjugate differ from neoplastic cells of different histopathological grades in gastric carcinoma.

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Introduction

Lectins are a group of specific glycoproteins present in animal and plant cells that specifically bind to terminal sugars of cell surface and extracellular matrix glycoconjugates. This ability of lectins to bind selectively to carbohydrate moiety of glycoproteins makes these proteins as differentiating markers to study cancers and metastatic cell lines (1).

This property of lectins depends on the process of cellular glycosylation. Glycosylation of some of the cell surface and extracellular matrix proteins and lipids control cell/ cell and cell extracellular matrix interactions (2). Incomplete glycosylation is considered to be the cause of carbohydrate alteration in cancer cells (2) Roman Snail lectin, Helix Pomatia agglutinin (HPA) is specific for N-acetylgalactosamine (GalNac) moiety of N-linked glycoprotein (3). New studies showed that

during neoplastic transformation, some compositional and structural changes have occurred in the oligosaccharide proportions of cell surface glycoproteins. This alteration result leads to new glycoproteins that did not exist before in the affected cells, or are reexpression of glycoconjugate which are characteristic of fetal live (4).

Although the incidence of gastric carcinoma is declining (5-6), it is a world wide disease and is the second most common cause of cancer mortality in the world (7). Gastric carcinoma is often not detected until an advanced stage and consequently the 5-year survival rate is 10-20% (5). Early detection of the gastric carcinoma by follow up of the patients with premalignant lesions have improved the survival rate up to 80% and should allow successful treatment of the neoplasia. Although Japanese studies demonstrated increased survival rate with low morbidity and post

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operation mortality, multicenter randomized control studies were unable to reproduce the results (6). Knowledge about the molecular features of gastric carcinoma has increased rapidly and researchers try to identify molecular markers that allow classification of gastric carcinoma with respect to important clinicopathological parameters (5). Abnormal glycosylation of cancer cells is a common feature of neoplastic cells that is responsible for anaplastic changes and altered biological properties of cancer cells (1). Studies of lectin histochemistry in tumor pathology provide some promising results (8). It seems that simultaneously with morphological changes of cancer cells such as nuclear pleomorphism, cellular atypia, and hyperchromasia and disorganized architecture, lectin staining properties of cancer cells were also changed (9). The present study was undertaken to identify the presence of GalNanc bearing glycoconjugate in cancer cells of different grades of gastric carcinoma and to compare the lectin staining properties of neoplastic cells in comparison with each other.

Patients and Methods

Formalin fixed and paraffin embedded tissue samples from a total number of 30 patients of gastric adenocarcinoma (10 patients for each grade) were collected from pathology file of Ali-Ebne-Abitaleb Hospital in Zahedan during 2005-2007. Prepared 5-7 micrometer sections were stained by hematoxylin and eosin (H&E) and were graded histopathologically by two expert pathologists (well differentiated, moderately differentiated and poorly differentiated adenocarcinoma). Parallel sections from each patients were stained by HPA/Alcian blue pH=2.5. Prepared sections were deparaffinized and hydrated according to routine procedures. HPA lectin (Sigma, USA) was diluted up to 10µg/ml with 0.1 M phosphate buffer solution (PBS pH=6.8). Prior to incubation with lectin, sections were treated for 5 minutes in a solution of 2% H₂O₂ in methanol, and then were incubated in humidified chamber for 2 hour with lectin in room temperature. After careful rinsing in PBS for 30 minutes, sections were immersed for 30 minutes in a 0.03% solution of diaminobenzidine (DAB) which contain 0.1% H₂O₂. Thereafter sections were carefully rinsed for 30 minutes with tap water and counterstained with Alcian blue pH=2.5 and then dehydrated, cleared and mounted according to routine procedures. Parallel control sections for each patient were used undergoing the same treatment procedure except for not incubating

with lectin or DAB. Histochemical grading according to staining intensity was blindly assigned in three microscopic fields as 3+ high reactivity, 2+ intermediate reactivity, 1+ low reactivity and 0 No reactivity]. Collected data were analyzed by Non Parametric Tests of Kruskal- Wallis and Mann-Whitney via SPSS software (Ver.13). Histopathological reports were prepared and photomicrography was done using Axiphot Zeiss® photomicroscope.

Results

Three histological grades of gastric carcinoma were determined according to glandular formation, nuclear atypia, hyperchromasia and rate of mitotic figure. In well differentiated adenocarcinoma, cancer cells formed well glandular structures, although the N/C ratio was increased. In poorly differentiated adenocarcinoma, cancer cells formed diffusely cords of neoplastic cells. In moderately differentiated adenocarcinoma, cancer cells show a very little preponderance to form glandular structures. Tumor stroma showed some degree of infiltrative inflammatory cells. The reactions of cancer cells in well differentiated adenocarcinoma to HPA lectin were high especially in apical plasmalemma and supranuclear portion of the cytoplasm (Figure 1).

In moderately differentiated adenocarcinoma cancer cells showed only intermediate diffuse cytoplasmic reaction to lectin (Figure 2).

In poorly differentiated adenocarcinoma, cancer cells showed heterogeneous pattern of reactivity in cytoplasm and nuclei (Figure 3).

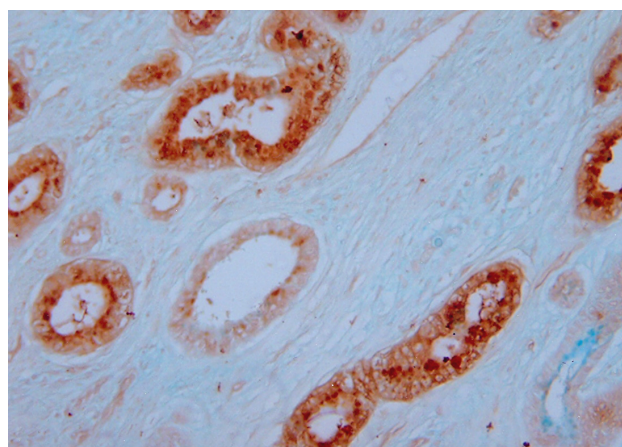
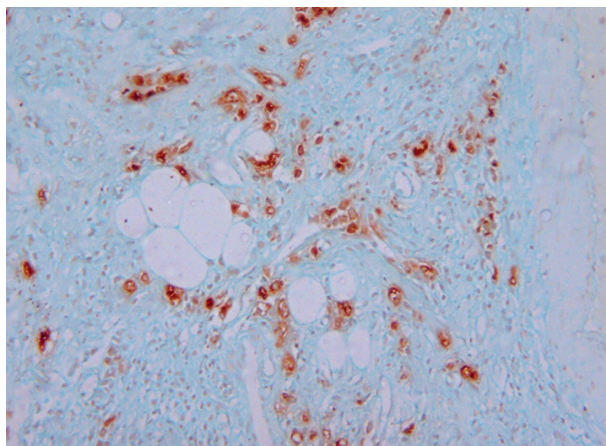


Figure 1. Staining properties of well differentiated gastric adenocarcinoma to HPA lectin was shown. HPA/Alcian blue pH=2.5 ×20

Table 1. Statistical parameters of HPA lectin staining reactivity of gastric cancer cells according to histopathological grading

Grades	HPA Reactivity	Mean	Mode
Well differentiated		2.04	3
Moderately differentiated		1.7	3
Poorly differentiated		2.1	3

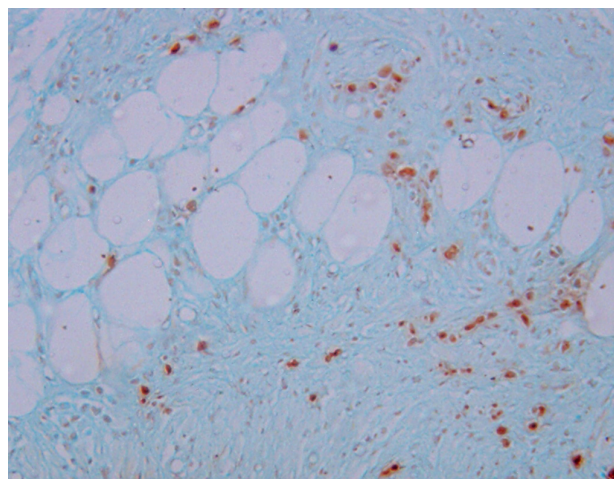
**Figure 2.** Staining properties of moderately differentiated gastric adenocarcinoma to HPA lectin was shown. HPA/Alcian blue pH=2.5 $\times 20$

In all grades of gastric adenocarcinoma tumor stroma did not show any pattern of reactivity to lectin (Table 1).

Statistical analysis of obtained data according to severity of staining to HPA lectin (i.e. showing the presence of GalNac disaccharide in glycoconjugate) revealed that there was a statistical difference between three histopathological grades of gastric adenocarcinoma (Kruskal- Wallis $P < 0.001$). Further analysis by Mann-Whitney test showed that this difference to staining properties of cancer cells is belonging to grade II (Moderately differentiated) and Grade III (poorly differentiated) $P < 0.01$. Furthermore there was a special pattern of heterogeneity in staining properties between cancer cells in different grades of gastric carcinoma (Table 2).

Table 2. Distribution of cancer cells histochemical reactivity to HPA lectin according to histopathological grading of gastric adenocarcinoma.

Histochemical grading	No Reactivity	Low reactivity	Intermediate reactivity	High reactivity
Histopathological grading	-	+	++	+++
Well differentiated	4.7%	24.5%	32%	38.6%
Moderately differentiated	18.8%	14.4%	37.6%	28.9%
Poorly differentiated	10.8%	15.6%	18%	55.4%

**Figure 3.** Staining properties of poorly differentiated gastric adenocarcinoma to HPA lectin was shown. HPA/Alcian blue pH=2.5 $\times 20$

Discussion

The incidence and mortality rate of cancer is still unacceptably high and hence the study of biology of glycoconjugates as the vital components of cell surface glycoprotein has been the field of extensive investigation. Altered glycosylation of glycoconjugate is one of the important changes that accompany malignant transformation (10). Glycosylation means the posttranslational modification of cell surface glycoprotein which can modify their functions (11).

Abnormal glycosylation of cancer cells may lead to synthesis of abnormal proteins in cell surface or cytoplasm that enable them invasive properties (9.). Our results showed different staining properties between cancer cells in all grades of gastric adenocarcinoma, which can show aberrant glycosylation in glycoconjugates of gastric carcinoma. Statistical analysis with nonparametric test of Kruskal Wallis showed that this difference in lectin staining properties for gastric carcinoma was significant ($P < 0.002$).

In accordance with our results, Bruchell *et al.* (2001) showed that lectin staining properties of cancer cells especially of invasive and metastatic cell lines may be relevant to anaplastic changes of cancer cells or may reflect the different biological behavior of tumor cells (12). The study of Fukotomi *et al.* (1991) showed that simultaneously with anaplastic changes of cancer cells, lectin staining properties were also changed in neoplasia (9). In accordance with this concept, our results showed that there was a significant difference between staining properties of moderately and poorly differentiated carcinoma ($P < 0.02$). Finding out the timing of the morphological changes of cancer cells and changes in lectin staining properties in cancer cells and its relationship to tumor progression and metastasis might be helpful in diagnosis, prognosis and treatment of cancer patients (13). Studies showed that there is a good association between gene amplification and carbohydrate structure in cancer. The positive rate of HPA breast cancer cells was related to c-myc gene amplification (9). The study of Noesett *et al.* (2004) showed that genetic changes in gastric carcinoma include amplification of c-erbB2 gene, mutation of ras, APC and p53 genes (5). It is probable that different lectin staining properties in gastric carcinoma may be relevant to these genetic alterations. Gastric carcinoma is often not detected until an advanced stage and hence knowledge about molecular changes of gastric carcinoma may be helpful for its molecular classification (5).

In accordance with our study, lectin histochemical techniques have shown some alteration in glycoconjugate of normal, benign and malignant gastrointestinal tumor. It is well known that there was a different pattern of lectin staining properties between cancer cells, which include plasmalemma, cytoplasmic and nuclear reactivity (8). Our results showed that there was a different pattern of HPA reactivity between cancer cells in all grades of gastric carcinoma. The predominant location of reaction to HPA lectin in well differentiated carcinoma was plasmalemma in comparison to diffuse cytoplasmic reaction in moderately differentiated and nuclear as well as cytoplasmic reaction in poorly differentiated gastric carcinoma. It is well known that HPA positive cancer cells have a more potential for metastasis than HPA negative cells in lung, breast, colon and gastric carcinomas. HPA has specificity for N-acetylgalactosamine and this saccharide is part of blood group A carbohydrate determinant. The molecular basis why HPA binds preferentially to metastasizing cancer

cells has not been elucidated completely (3). It is probable that GalNac containing glycoconjugate may have different roles in glandular epithelia than stratified epithelia in gastrointestinal tract (3). Although Maccartney *et al.* (1986) showed that there was no correlation between lectin staining properties and the stage or differentiation of gastric carcinoma (14), our results showed that there was a good correlation between staining properties of cancer cells especially in moderately and poorly differentiated gastric adenocarcinoma. It is well known that difficulties in interpreting histochemical demonstration of lectin binding sites may be attributable to different techniques and masking properties of sialic acid (3, 15). Okoyama *et al.* (1998) showed that there was a correlation between HPA binding properties of gastric cancer cells and immunologic status of the patients (16). It seems that different lectin staining properties of cancer cells may provide good knowledge about biological properties of cancer cells in gastric carcinoma.

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