Research Article

Immunohistochemical expression of galectin-3 in colorectal Adenocarcinoma

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Abstract
Background: colorectal cancer is one of the most common cancers worldwide. It is a result of complex interaction of genetic mutations and environmental factors. Galectin-3 is expressed in a wide range of normal and neoplastic tissues and has emerged as an important regulator of diverse functions critical in cancer biology including cellular adhesion, proliferation, apoptosis, metastasis, immune surveillance and gene expression. **Methods:** immunohistochemical staining of galectin-3 was conducted on 60 tissue specimens of colorectal adenocarcinoma by using the avidin biotin-peroxidase complex method with diaminobenizidne (DAB) chromagen detection system. **Results:** High galectin-3 expression was detected in 65% of cases. Galectin-3 expression showed statistically significant correlation with lymph node metastasis (p = 0.036) and advanced tumor stage assessed by both TNM staging (p= 0.014) and modified dukes staging (p=0.035). **Conclusions:** galectin-3 expression is significantly associated with poor prognostic factors and thus can be used as a prognostic indicator for colorectal carcinoma patients.
Keywords: galectin-3, colorectal carcinoma, immunohistochemistry.

Introduction
Colorectal cancer (CRC) is the third most common cancer worldwide and a leading cause of mortality and morbidity, representing a major public health problem(Torre et al., 2015). In Egypt, the colonic cancer incidence in Egyptian males is 2.63% while in females is 2.28% among cancers. Rectal cancer incidence in Egyptian males is 0.84% while in females is 0.72% among cancer (Ibrahim et al., 2014).

Galectin-3 present at the cell surface and in the extracellular space binds to its numerous extracellular binding sites present in several ligands such as integrin's, mucins, and growth factor receptors. Galectin-3 is therefore involved in many carbohydrate- mediated processes such as cell adhesion, cell-cell interaction, cell migration and cell signaling, and is a proapoptotic stimulus to T-cells (Vanderstraeten et al., 2014).

In colorectal carcinoma, galectin-3 expression was still a matter of debate (Nagy et al., 2003) as some investigators found decreasing galectin-3 levels in CRC progression (Tsuboi et al., 2007; Ibrahim et al., 2015) whereas other studies have demonstrated an increased expression (Fathy, 2013; Huang et al., 2016).

Experimental studies have revealed that there was less tumor burden and metastasis in the MCP- fed nude mice into which human colon carcinoma cells were implanted than in the control mice (Nangia-Makker et al., 2002).

Material and Methods
1. Tissue specimens
The present study comprised 60 randomly selected cases of primary colorectal adenocarcinomas. Tumor type and grade were evaluated according to WHO criteria (Hamilton et al., 2010). Tumor stage was estimated by TNM staging (Edge and Compton, 2010) and modified Dukes staging (Li et al., 2013).

2. Immunohistochemistry:
Primary antibody against galectin-3: polyclonal mouse antibody (100µ, concentrated, lab vision laboratories), diluted at (1;300). For negative control the primary antibody was PBS. Positive control was normal rat colon.

3. Scoring of Immunostaining:
Galectin-3 was expressed mainly in the cytoplasm. Ten high power fields were counted per section in each case and the average of counted fields was calculated. The median was taken as a cut off point for galectin-3 expression, and the expression was divided into 2 categories; low expression and high expression. For statistical purposes, negative cases are included among low expressions and considered as on category.

Statistical analysis:
Statistical analysis was conducted using the statistical package for social (SPSS software version 16).

Results

clinic pathological Features:
Data regarding different clinical and histopathological features for colorectal adenocarcinoma patients are summarized in Table(1).

Clinicopathological features	No. (%)
Age	
<50	26(43.3%)
≤50	34(56.7%)
Gender	
Male	32(53.3%)
female	28(46.7%)
Location	
Colon	44(73.3%)
Rectum	16(26.7%)
Tumor size	
<5	24(40%)
≥5	36(60%)
Histological subtypes	
Adenocarcinoma	46(76.7%)
Mucinous carcinoma	12(20%)
Signet ring cell carcinoma	2(3.3%)
Tumor grade (adenocarcinoma)*	
Low grade	36(78.3)
High grade	10(21.7)
Nodal status	
Negative	32(53.3%)
positive	28(46.7%)
TNM stage	
I	6(10%)
II	26(43.3%)
III	28(46.7%)
Modifiend Dukes stage	
B1	6(10%)
B2	26(43.3%)
C1	8(13.3%)
C2	20(33.4%)
Tumor infiltrating lymphocytes	
Abest	4(6.7%)
Mild	27(45%)
Moderate	12(20%)
marked	17(28.3%)

*Varible specific for conventional adenocarcinoma only.

Immunohistochemical expression of cytoplasmic galectin-3 and its association with patients' clinicopathological features

Table (2): Association between cytoplasmic galectin-3 expression and clinicopathological features for patients with CRC (n=60)

Clinicopathological feat	Total 60 (100%)	Cytoplasmic galectin-3 expression		P value
		Negative/low Expression 21(35%)	High expression 39(65%)	
Age				
<50	26(43.3)	11(42.3)	15(57.7)	0.222
≥50	34(56.7)	10(29.4)	24(70.6)	
Gender				
Male	32(53.3)	12(37.5)	20(62.5)	0.436
female	28(46.7)	9(32.1)	19(67.9)	
Location				
Colon	44(73.3)	17(38.6)	27(61.4)	0.254
Rectum	16(26.7)	4(25%)	12(75)	
Tumor size				
<5	24(40)	7(29.2)	17(70.8)	0.311
≥5	36(60)	14(38.9)	22(61.1)	
Histological subtypes				
adenocarcinoma mucinou	46(76.7)	11(23.9)	35(76.1)	
carcinoma signet	12(20)	8(66.7)	4(33.3)	0.003*
ring cell carcinoma	2(3.3)	2(100)	0(0)	
Tumor grade (adenocarcinoma)*				
Low grade	36(78.3)	9(25)	27(75)	
High grade	10(21.7)	2(20)	8(80)	0.553
Nodal status				
Negative	32(53.3)	15(46.9)	17(53.1)	0.036*
positive	28(46.7)	6(21.4)	22(78.6)	
TNM stage				
I	6(10)	5(83.3)	1(16.7)	
II	26(43.3)	10(38.5)	16(61.5)	
III	28(46.7)	6(21.4)	22(78.6)	0.014*
Modified dukes stage				
B1	6(10)	5(83.3)	1(16.7)	
B2	26(43.3)	10(38.5)	16(61.5)	
C1	8(13.3)	2(25)	6(75)	0.035*
C2	20(33.4)	4(20)	16(80)	
Tumor infiltrating lymphocytes				
Absent	4(6.7)	2(50)	2(50)	
Mild	27(45)	8(29.6)	19(70.4)	0.808
Moderate	12(20)	5(41.7)	7(58.3)	
Marked	17(28.3)	6(35.3)	11(64.7)	

*p-value <0.05 are considered statistically significant according to Chi-Square test and Fisher's exact test.
*Variable specific for conventional adenocarcinoma only.

In the current study, a statistically significant association was detected between cytoplasmic galectin-3 expression and the histological type in the studied cases (p=0.003). also, a statistically significant positive association was detected with lymph node metastasis (p=0.036) and advanced tumor stage assessed by both TNM staging(p=0.014) and modified Dukes staging (p=0.035).

Discussion

In the present study, 65% of cases showed high cytoplasmic galectin-3 expression, while 35% showed negative/low expression. Fathy et al., (2013) and Tao et al., (2017) reported similar results. Ibrahim et al., (2015) reported higher galectin-3 expressions (81.7%), while others detected lower expressions ranging from 42.4% to 51% (huang et al., 2016).

On studying cytoplasmic galectin-3 expression in relation to different clinicopathological features, a statistically significant difference in galectin-3 expression was detected among different histological subtypes and this coincides with what was noted by Mahmoud et al., (2011) and Ibrahim et al., (2015). However, this was in contrast to what was reported by (gopalan et al., (2016) who found no significant difference.

In the current study, a statistically significant positive association between cytoplasmic galectin-3 expression and regional lymph node involvement was detected where 78.6% of cases positive for lymph node metastases showed high expression. This was in accordance with (Endo et al., 2005; Gopalan et al., 2016) who detected higher expressions in cases positive nodal; metastasis. However, this was in opposite to what was reported by Ibrahim et al., (2015) and petrovic et al., (2016) who found non-significant association.

A statistically significant positive association was observed also between cytoplasmic galectin-3 expression and advanced tumor stage assessed by TNM staging where the majority of III cases (78.6%) showed high expression while 61.5% of stage II cases and 16.7% of stage I cases showed high expression. This was in line with findings of (Endo et al., 2005; Huang et al., 2016) who reported significant higher expression rate with advanced tumor stage, but in contrast to what was reported by Tsuboi et al., (2007) who observed decreased galectin-3 expression in advanced stages.

Concerning modified Dukes classification, Was also detected a statistically significant positive association where 16.7% of stage B1 cases and 61.5% of stage B2 cases showed high expression while 75% of stage C1 cases and 80% of stage C2 cases showed high expression. tis finding was consistent with other studies (Endo et al., 2005; Fathy, 2013; Tao et al., 2017) who reported significant higher expr-essions in dukes B and C cases .

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