Clinicopathologic Significance of Galectin-3 and Glucose Transporter 1 Expressions in Colorectal Cancer

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Abstract: Background: Colorectal cancer (CRC) is the second and third most common cancer in women and men respectively. The molecular prognostic factors that may provide targeted therapy to improve the clinical outcome of patients with colorectal adenocarcinomas would be of great help for patients who are likely to benefit from adjuvant therapies and improve their prognosis. **Aim of the work:** To investigate the prognostic value of galectin-3 and glucose transporter 1 (GLUT1) expressions in colorectal carcinoma and their relationship to clinicopathological parameters. **Methods:** Galectin-3 and GLUT1 expressions were evaluated using immunohistochemical staining in 50 patients with colorectal cancer. The relationship between their expressions and clinicopathological factors were analyzed. **Results:** Galectin-3-positive expression was detected in 44 patients (88%). The incidence of lymph node and distant metastasis in galectin-3 positive cancer was significantly higher than that in galectin-3-negative cases (P =0.001 and P =0.015, respectively). Furthermore, galectin-3 immunoreactivity was significantly associated with tumor size (P =0.001). GLUT1 was expressed in 84% of patients with CRC. GLUT1 expression was significantly correlated with non-mucinous tumor type (P =0.018), poorer differentiation (P = 0.011), lymph node metastasis (P < 0.039), and higher TNM stage (P < 0.007). **Conclusion:** Galectin-3 and GLUT1 expressions were frequently increased in colorectal adenocarcinomas and significantly associated with poorer clinicopathologic phenotypes.
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Key words: Colorectal carcinoma, Galectin-3, GLUT1, Prognosis, clinicopathologic.

1. Introduction

Worldwide, colorectal cancer (CRC) is the second most common cancer in women (9.2% of all cancers) and the third most common in men (10.0% of the total)[(1)]. In Egypt, according to National cancer institute (NCI), cancer pathology registry 2003-2004; CRC accounts for 4.34% of all cancers. It occupied the fourth rank among male and female malignancy[(2)], but nowadays colon cancer was (2.91% and 2.31% in 100,000 populations) in male and female respectively[(3)].

Patients with colorectal cancer, except for the advanced stage, undergo curative resection. Stage II patients with obstruction, perforation or certain tumor markers, and stage III patients, will take adjuvant chemotherapy after surgical resection. The molecular prognostic factors that may provide targeted therapy to improve the clinical outcome would be of great help for patients who are likely to benefit from adjuvant therapies and improve their prognosis[(4)].

Galectin-3 is a 31-kDa gene product that serves as an intracellular and extracellular lectin and that is thought to interact with glycoproteins located on the cell surface matrix[(5)]. Galectin-3 has important roles in diverse biological events, such as embryogenesis, cell growth and adhesion, proliferation, differentiation, cell-cycle progression, apoptosis, mRNA splicing, and regulation of the immune system[(6)].

Previous studies have reported an enhancement of glycolytic metabolism in tumors. Increased glucose uptake and use is essential for many malignant tumors. This process is mediated by the glucose transporters (GLUTs) which are membrane proteins responsible for the transport of glucose across cell membranes. GLUT1 is an isoform of seven cloned glucose transporters that is expressed in erythrocytes. GLUT1 protein expression can be altered by a number of factors, including cellular differentiation and transformation, and also can be altered under the influence of growth factors, insulin, glucose, and even stress[(7,8)]. The increased expression of GLUT1 mRNA and protein has been demonstrated in various cancer tissues which indicate that GLUT1 may play an important role in glucose uptake by various cancers and that GLUT1 expression could be a useful marker for malignant transformation[(9)].

In this study, the immunohistochemical expressions of galectin-3 and GLUT1 were investigated in series of 20 normal mucosa and 50 colorectal adenocarcinomas. It was also a trial to evaluate the expressions of these two biomarkers in colorectal carcinogenesis, and their relationship to clinicopathological parameters.

2. Materials & methods

Tissues and patient history

For this retrospective cohort study, formalin-fixed paraffin embedded tissue samples from 50 patients with colon cancer (39, 6 and 5 cases of adenocarcinomas, mucinous carcinoma and adenocarcinoma with mucinous component less than 50%, respectively). Twenty paraffin blocks from normal colonic mucosa adjacent to the carcinoma and from patients subjected to diagnostic colonoscopy were enrolled in this study. The tissue blocks were collected from the archives of Pathology Department, Faculty of Medicine, Zagazig University between 2011 and 2014. The TNM classification was used for pathologic staging, and the World Health Organization classification was used for pathologic grading[10]. The study complied with the guidelines of the local ethics committee.

Immunohistochemical staining

Multiple 4 µm sections were cut, then transferred to positively charged slides. Slides were deparaffinized in xylene and rehydrated in graded alcohol. Sections were boiled in citrate buffer (pH 6.0) for 20 min. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxidase for 20 min. The slides were then incubated overnight with monoclonal antibodies; GLUT1 antibody (ab40084, dilution 1:100 Abcam, Cambridge, UK) and galectin-3 antibody (clone 3Ab1 (9C4) dilution 1:80 Neo Markers, Lab Vision Corporation, Fremont, CA, USA). After three washes, 2 min each with PBS, the sections were incubated with a biotinylated goat anti-mouse secondary antibody and product visualization (Lab Vision Corporation, Fermont, USA). After three washes, 2 min each, the samples were developed with diaminobenzidine substrate as a chromogen for 1 min and counterstained with Mayer's hematoxylin. Negative controls were performed by omitting the galectin-3 and GLUT1 anti-bodies during the primary antibody incubation. Normal colon mucosa and erythrocytes were used as positive control for galectin-3 and GLUT1, respectively.

Assessment of immunohistochemistry

GLUT1 immunostaining in colorectal carcinoma was quantitated by grading the proportion of cells that were GLUT1 positive as (<10%, 10–50%, or >50; low, moderate and high grade respectively). For purposes of statistical analysis, a cut-off value of 50% was accepted[11].

Galectin-3 expression, if the percentage of positive-staining cancer cells accounted for less than 20% of the total number of cancer cells, the staining was defined as negative and if more than 20%, staining was defined as positive[12].

Statistical Analysis

Statistical analysis was performed using SPSS software (SPSS, Chicago, version 20 IL, USA). Data were expressed as mean ±SD for quantitative variables. Fisher's exact test or chi-square was used to analyze the distribution of markers among different groups. The *P*-value less than 0.05 was considered significant.

3. Results

Fifty patients, 30 males and 20 females were enrolled in this study, with age ranged from 34-78 years (mean: 58 ±11.8). The demographic data of our patients were shown in table (1).

Galectin-3 expression

Galectin-3-positive expression was detected in 44 patients (88%) with CRC. Its expression decreased in the mucinous component of the tumor when compared with the normal mucosa and to non-mucinous carcinoma (P <0.001). The incidence of lymph node and distant metastasis in galectin-3 positive cancer was significantly higher than that in galectin-3-negative cases (P =0.001 and P =0.015, respectively). Furthermore, galectin-3 immunoreactivity was significantly associated with tumor size (P =0.001). There was no statistically significant difference in galectin-3 expression as regarding gender, age, tumor grade and tumor site, (Table 1, Figs.1-6).

GLUT1 expression

All 20 normal mucosa specimens (100%) were negative for GLUT1 expression. However, in colorectal adenocarcinomas, 18 (36%) of 50 cases revealed a high grade of GLUT1 expression and 16 cases (32%) and 8 cases (16%) showed moderate grade and low grade of GLUT1 expression, respectively. Only 8 (16%) of 50 cases were negative for GLUT1 expression (**P = 0.0001).**

There was significant association between the degree of GLUT1 immunostaining and the histologic differentiation of the tumors P = 0.011 (high expression in poorly differentiated tumor). We found higher GLUT1 expression correlated with frequent lymph node metastasis and non mucinous tumor P = 0.039 and 0.018 respectively. The association of GLUT1 staining status with TNM stage was statistically significance P = 0.007. GLUT1 staining more than 50% was associated with the presence of distant metastases (P =0.033). There was no statistically significant relation between GLUT1 expression and gender, age, tumor site and tumor size, (Table 2, Figs. 7- 10).

Table (1): Galectin-3 expression in relation to clinicopathological parameters

Clinicopathological variants	No	%	Galectin-3 expression				χ^2	*P*
			+ve (n =44)		-ve (n=6)			
			No	%	No	%		
Gender								
Male	30	60%	26	86.7%	4	13.3%	0.007	0.929
Female	20	40%	18	90%	2	10%		
Age								
<50	16	32%	12	75%	4	25%	2.173	0.140
≥50	34	68%	32	94.1%	2	5.9%		
Tumour site								
Right	20	40%	19	95%	1	5%	1.726	0.421
Left	8	16%	7	87.5%	1	12.5%		
Rectum	22	44%	18	81.9%	4	18.1%		
Tumour size								
<5	12	24%	6	50%	6	50%	17.116	**<0.001***
≥5	38	76%	38	100%	0	0%		
Lymph node metastasis								
Negative	16	32%	10	62.5%	6	50%	11.155	**<0.001***
Positive	34	68%	34	100%	0	0%		
Histological types								
Adenocarcinoma (NMC)	39	78%	39	100%	0	0%	26.010	**<0.001***
Mucinous carcinoma component (MCC)	5	10%	3	60%	2	40%		
Mucinous carcinoma (MC)	6	12%	2	33.3%	4	66.7%		
Grades								
Grade I	24	48%	24	100%	0	0%	2.642	0.266
Grade II	12	24%	12	100%	0	0%		
Grade III	14	28%	8	57.1%	6	42.9%		
TNM stage								
I, II	16	32%	11	68.7%	5	31.2%	8.316	**0.015***
III	28	56%	27	96.4%	1	3.6%		
IV	6	12%	6	100%	0	0%		
Total	50	100%	44	88%	6	12%		

*** Significant *P* value.**

Table (2): GLUT1 expression in relation to clinicopathological parameters:

Clinicopathological variants	No	%	GLUT1 expression				χ^2	*P*
			<50% (n =32)		≥50 (n=18)			
			No	%	No	%		
Gender								
Male	30	60%	20	66.7%	10	33.3%	0.213	0.644
Female	20	40%	12	60%	8	40%		
Age								
<50	16	32%	11	68.8%	5	31.2%	0.027	0.869
≥50	34	68%	21	61.8%	13	38.2%		
Tumor site								
Right	20	40%	13	65%	7	35%	0.017	0.991
Left	8	16%	5	62.5%	3	37.5%		
Rectum	22	44%	14	63.6%	8	36.4%		
Tumor size								
<5	12	24%	8	66.7%	4	33.3%	0.015	0.901
≥5	38	76%	24	63.2%	14	36.8%		
Lymph node metastasis								

Negative	16	32%	14	87.5%	2	12.5%	4.240	**0.039***
Positive	34	68%	18	52.9%	16	47.1%		
Histological types								
Adenocarcinoma (NMC)	39	78%	21	53.8%	18	46.2%	7.933	**0.018***
Mucinous carcinoma component (MCC)	5	10%	5	100%	0	0.0%		
Mucinous carcinoma (MC)	6	12%	6	100%	0	0.0%		
Grades								
Grade I	24	48%	20	83.3%	4	16.7%	8.922	**0.011***
Grade II	12	24%	7	58.3%	5	41.7%		
Grade III	14	28%	5	35.7%	9	64.3%		
TNM stage								
I, II	16	32%	14	87.5%	2	12.5%	9.801	**0.007***
III	28	56%	17	60.7%	11	39.3%		
IV	6	12%	1	16.7%	5	83.3%		
Metastasis								
M0	44	88%	31	70.5%	13	29.5%	4.501	**0.033***
M1	6	12%	1	16.7%	5	83.3%		
Total	50	100%	32	64	18	36		

*** Significant *P* value.**

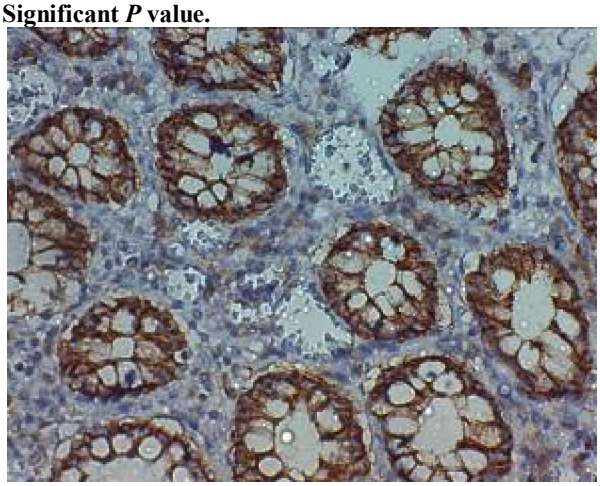

Fig (1) Galectin-3 immunostaining of normal colon showing membranous expression (x200)

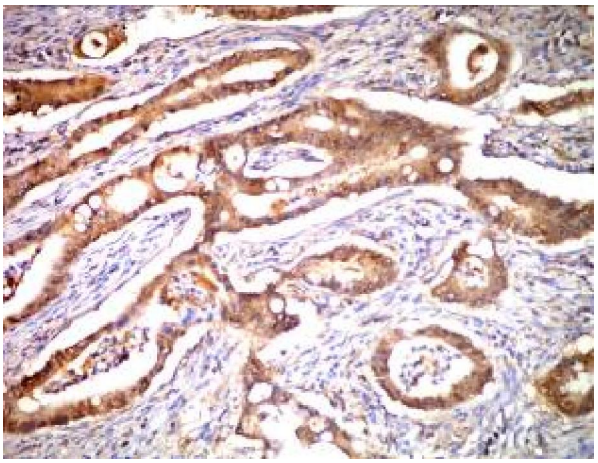

Fig (2) Galectin-3 immunostaining of well differentiated colonic adenocarcinoma showing cytoplasmic expression (x200)

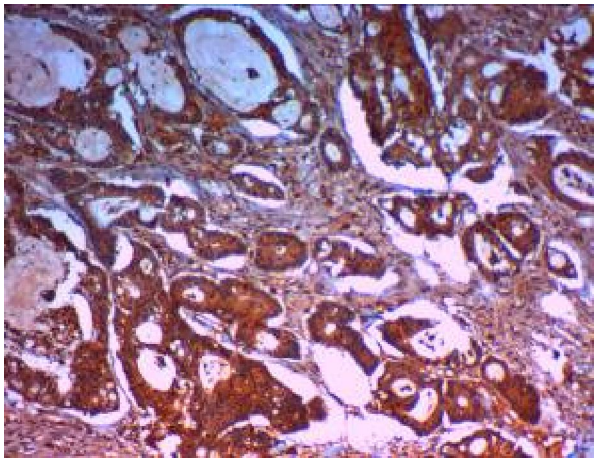

Fig (3) Galectin-3 immunostaining of moderately-differentiated colonic adenocarcinoma showing cytoplasmic expression (x200)

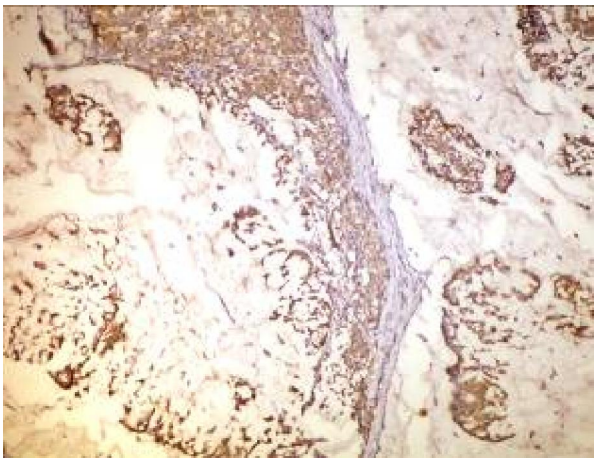

Fig (4) Galectin-3 immunostaining of mucinous adenocarcinoma in the colon showing cytoplasmic expression (x200)

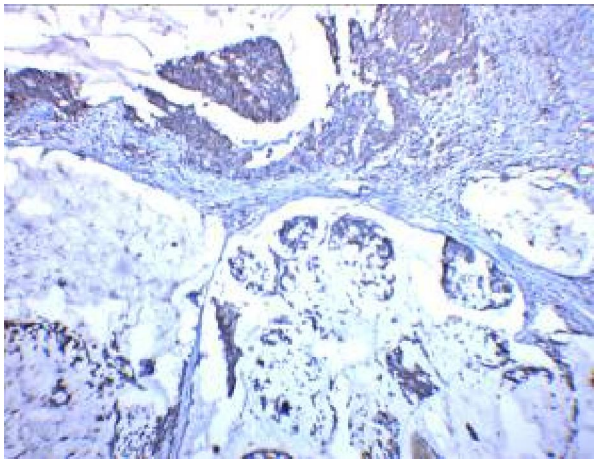

Fig (5) Galectin-3 immunostaining of mucinous carcinoma component of CRC showing negative expression (x200)

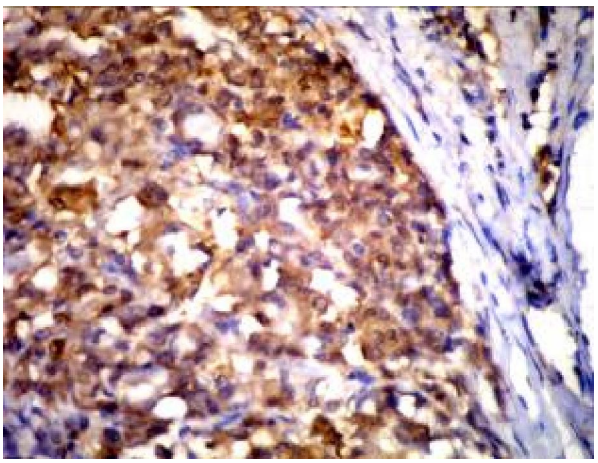

Fig (6) Galectin-3 immunostaining of poorly differentiated adenocarcinoma in the colon showing cytoplasmic expression (x400)

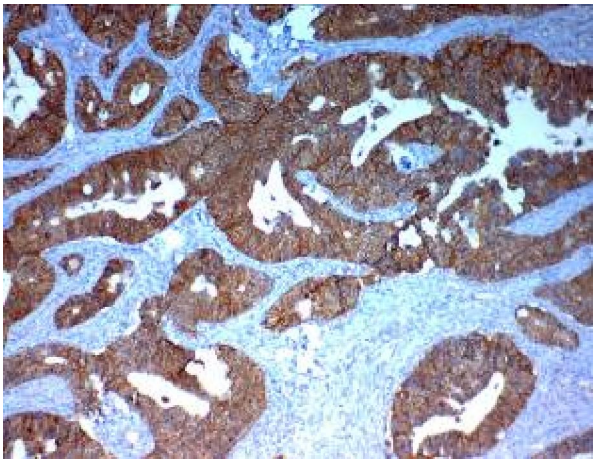

Fig (7) GLUT1 of well differentiated adenocarcinoma of the colon showing membranous expression (>50%) of carcinoma cells (x200)

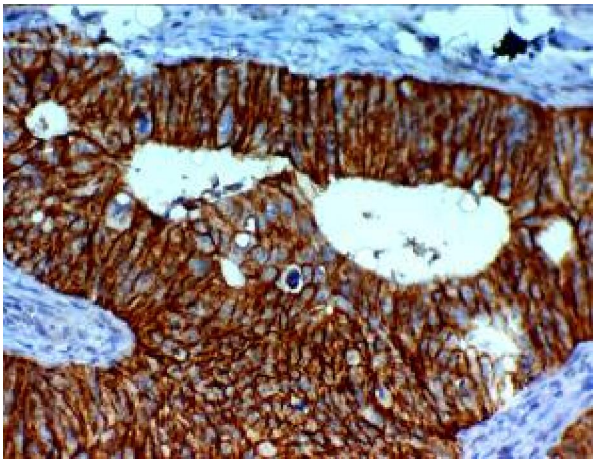

Fig (8) GLUT1 of moderately differentiated adenocarcinoma of the colon showing membranous expression (>50%) (x400)

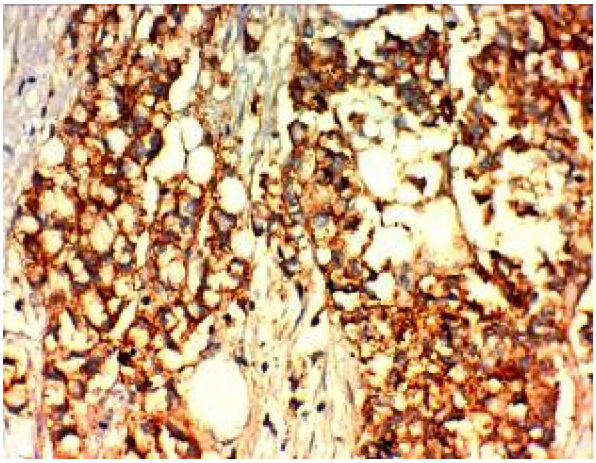

Fig (9) GLUT1 of poorly differentiated adenocarcinoma of the colon showing membranous expression (x400)

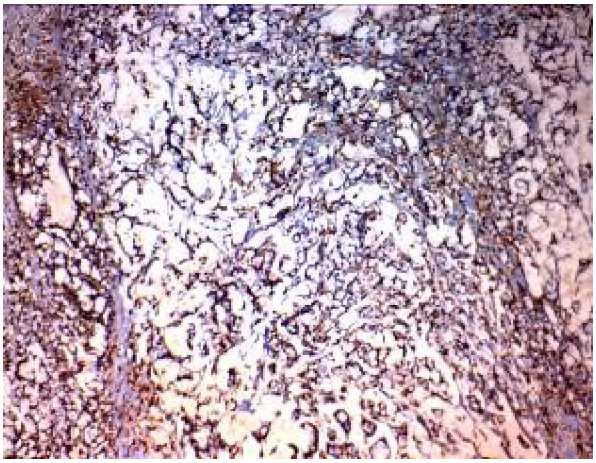

Fig (10) GLUT1 of mucinous adenocarcinoma of the colon showing membranous expression (<50%) (x200)

4. Discussion

Tumor stage of colorectal cancer is the best prognostic factor and best predictor of survival after surgical resection. However, patients diagnosed stage II colorectal cancer (CRC), up to 35% will undergo recurrence[13]. Several molecular biomarkers have been proposed as markers of poor prognosis in stage II patients[14,15].

Diagnostic implications of galectin-3 expression in malignancies including thyroid, pulmonary and colon cancer were stated [12,16,17]. Meanwhile, several studies have shown that decreased expression of galectin-3 is associated with malignancies including colorectal cancer [18,19]. This shows that inconsistent and varying amounts of galectin-3 immunoreactivity in tumors of the same origin reflect tumor cells heterogenicity. Therefore, further studies to elucidate the biological significance of galectin-3 expression in colorectal cancer are needed.

In the present study, galectin-3 was highly expressed in colorectal cancer; it was expressed in 88% of cases. These findings are in agreement with, **Zaia povegliano *et al.*[12], Endo *et al.*** [20] and **Shoeppner *et al.*** [21] who reported that 57.3%, 65% and 97% of colorectal cancer were positive, respectively. In contrast, other studies reported decreasing galectin-3 levels in colon progression[18,19]. This discrepancy can be due to complex processes that regulate galectin-3 expression; it may depend on cell type, external stimuli, environmental conditions and numerous transcription factors and signaling pathways.

Galectin-3 changes its subcellular localization from nucleus to the cytoplasm during progression from colorectal adenoma to carcinoma. As cytoplasmic galectin-3 is known to be an apoptosis inhibitor, so change in cellular localization may contribute to cancer cell survival. Also galectin-3 inhibits CD95/Fas-mediated caspase-8 activation and apoptosis[22].

We also found a decrease of galectin-3 in mucinous carcinoma and adenocarcinoma with mucinous component less than 50%, with decrease or even a complete absence of galectin-3 in mucinous areas. This finding goes with study of **Ben Mahmoud *et al.*** [23] that showed an expression of galectin-3 mRNA in nonmucinous carcinoma. However, mucinous carcinomas did not express. **Ben Mahmoud *et al.*** [23] stated that DNA methylation is responsible for silencing or reduced expression of gal-3 in colorectal mucinous carcinoma.

We declared that, galecin-3 expression was correlated with colorectal cancer metastasis (P =0.015). This is in agreement with **Bresalier *et al.*** [24] and **Hittelet *et al.*** [25]. Galectin-3 provides tumor cells with anti-apoptotic activities, which need for anchorage-independent cell survival in the circulation to disseminate[26]**.** Galectin-3 enhances cancer cell–cell and cancer cell–matrix interactions and promotes cancer cell spread to secondary sites, so high galectin-3 expression in CRC patients increase the risk of metastasis and it has been suggested to be a useful prognostic marker[27]

Zaia Povegliano *et al.* [20] have found that a high percentage of galectin-3 expression could be observed in advanced colon cancer patients and those with recurrence after surgery and chemotherapy treatment.

More than 90% of carcinogenesis in colorectal cancers begins with mutations in the β-catenin signaling pathway[28]. Galectin-3 silencing interrupted the β-catenin pathway and inhibited cyclin D1 and c-myc, which play critical roles in cell cycle regulation. **Lee *et al.*** [29] showed that galectin-3 silencing sensitizes MDR (multidrug resistance) cells to epirubicin by inhibiting p-glycoprotein and MDR-associated proteins and the activation of the mitochondrial apoptosis pathway through modulation of the β-catenin/GSK-3βpathway in human colon cancer cells.

SMAD4 is localized to chromosome 18q21, a major suppressor of colorectal cancer progression. SMAD4 deficiency was responsible for the enhanced migration of colon cancer cells with increase in matrix metalloprotease 9, enhanced hypoxia-induced GLUT1 expression, increased aerobic glycolysis, and resistance to 5′-fluoruracil–mediated apoptosis. GLUT1 is involved in colon cancer progression mediated by SMAD4 loss[30].

The expression of GLUT1 appears to be a potential marker for malignant transformation, and the degree of GLUT1 expression correlates with biologic behavior of tumor. The results of the current study confirm that the GLUT1 protein is expressed by tumor cells in the majority of cases (84%) of colorectal carcinoma, and also confirm the correlation between a higher degree of GLUT1 expression and the presence of lymph nodes and distant metastases. Increased GLUT1 expression in malignant tissue reflects an increased glycolytic metabolism and is showed under conditions that induce greater need for glycolysis, such as ischemia or hypoxia[7]**.** Some studies **Sakashita *et al.*** [7]**; Jun *et al.*** [31]**; Haber *et al.*** [11] have reported the correlation between GLUT1 expression and the clinicopathologic parameters in colorectal adenocarcinomas.

Cancer cell upregulated glucose transport and aerobic glycolysis regardless of their oxygen status. They are sensitive to glucose deprivation due to the dependence on glucose as a major nutrition supply. The increased glucose transport in cancer cells has been attributed primarily to the upregulation of glucose transporter 1 (Glut1) that are responsible for

basal glucose transport in almost all cell types[32]. Knockdown of Glut1 by shRNA chemo-sensitized head and neck cancer cells to cisplatin[33].PcGal16 a promising therapeutic agent for the treatment of bladder cancer acts as efficient anti-cancer photosensitizers through its ability to reduce GLUT1[34]. **Liu *et al.*** [35] reported that Glut1 inhibitors such as WZB117 may be considered an additional treatment options for patients with 5-Flurouracil resistant colon cancers.

Conclusion

Correlation between the expressions of galectin-3 and GLUT1 and the clinicopathologic parameters in colorectal adenocarcinomas could help to uncover novel biomarkers for advanced stage colon cancer. This might improve prognostic evaluations and identify effective targets for therapeutic intervention.

Abbreviations

CRC: Colorectal cancer; GLUT1: glucose transporter 1

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