The expression profiles of the galectin gene family in colorectal adenocarcinomas.

Running head: galectin gene family in colorectal cancer

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Conflict of interest

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Summary

We aim to investigate the expression profiles of galectin family genes (*galectins-1, 2, 3, 4, 7, 8, 9, 10 & 11*) in colorectal carcinomas. mRNA expression of *galectin* family members (1, 2, 3, 4, 7, 8, 9, 10 and 12) were analysed by real-time polymerase chain reaction in colorectal tissues from 201 patients (54 non-cancer colorectal tissues, 49 adenomas and 98 adenocarcinomas). Galectin-1 and galectin 3 protein expression was determined by immunohistochemistry. In general, high *galectin* mRNA expression was noted in colorectal carcinomas in early stages of their pathogenesis. Significant differences in *galectins-2, 3, 7, 8* and *10* mRNA expression was associated with pathological stages ($p < 0.05$). Increased prevalence of *galectins-2, 7, 8* and *10* mRNAs overexpression was noted in non-metastatic colorectal carcinomas ($p < 0.05$). Galectin-1 and galactin-3 proteins were present in the nucleus and cytoplasm of the colorectal tissues and expressed significantly higher in colorectal carcinomas when compared to colorectal adenomas (61% and 95% respectively). Patients with colorectal carcinoma with high levels of *galectin-3* mRNA and protein expression showed better prognosis ($p=0.052$). To conclude, many novel correlations between the deregulation of galectin family genes and various clinicopathological features in colorectal adenocarcinoma were noted. Overexpression of *galectins* at the mRNA level and proteins were predominant in earlier stages of colorectal carcinomas. These altered expression patterns of galectin genes suggest the multi-functional role of galectin genes in the regulation of colorectal cancer development, progression and metastasis.

Keywords: galectin; colorectal carcinoma; protein, mRNA.

Introduction

The galectins are a family of 15 mammalian galactoside binding proteins that share a consensus amino acid sequence in their carbohydrate recognition domains [1]. Altered expression of galectins has been implicated in cancer emergence and progression [2]. Among the galectin family genes, *galectin-1* and *galectin-3* have been proposed to have a significant role in cancer pathogenesis, modulating the cell-to-cell or cell-to-extra cellular matrix binding [3, 4]. Galectin-3 plays key roles in cell adhesion, cell growth and metastasis [5], whilst galectin-1 has been suggested to moderate the immune response and apoptosis [6]. In pathology practice, galectin 3 is a marker to help in the differentiation of papillary thyroid carcinoma from non-neoplastic thyroid lesions [7]. In colorectal cancer, galectin molecules have been reported to play a key role in the regulation of development, progression and metastasis of the cancer [8-9]. In this study, we aim to investigate the expression profiles of different *galectin* family genes (*galectin-1, 2, 3, 4, 7, 8, 9, 10 & 11*) in colorectal carcinomas with emphasis on galectin-1 and 3 protein expressions.

Materials and Method

Selection of patients

The patients chosen for this study had resection for primary colorectal adenomas (pre-invasive neoplasia), colorectal adenocarcinomas, metastatic colorectal adenocarcinomas in distant organs and non-neoplastic colorectal mucosae between 2005 and 2013 in Queensland, Australia. Patients were sequentially chosen and with no selection bias. Ethical approval of this study has been obtained from the Griffith University Human Research Ethics Committee (GU Ref No: MED/05/06/HREC).

Histological sections were cut and stained for haematoxylin and eosin (H&E) for microscopic examination. After histological review, tissues from 201 patients (54 non-neoplastic mucosae: 49 adenomas; 98 primary adenocarcinomas) were recruited for this study. In addition, matched primary and metastatic tissue samples were collected. These included 26 lymph nodes with metastatic colorectal adenocarcinoma as well as 15 resected livers with metastatic colorectal adenocarcinoma. Tissue blocks were checked to ensure that they contained a representative cancer area.

Clinical pathological parameters

The colorectal adenocarcinomas were classified and graded and according to the World Health Organisation criteria and staged according to TNM (Tumour, Lymph node and Metastases) classification [10]. Clinical and demographic data collected include age, gender of the patients and locations of the cancers. Pathological parameters analysed included size of the cancer, histological grade, presence of metachronous/synchronous cancers, associated polyposis, cancer perforation, lymphovascular invasion. Both conventional (n=84/98) and mucinous adenocarcinomas (n=14/98) (>50% mucin distribution in conventional adenocarcinomas) were included [11].

Clinical follow-up

The patients with colorectal adenocarcinoma were managed by a pre-agreed standardized protocol with the management of every patient being discussed in weekly multidisciplinary team meetings. Cancer recurrence or residual diseases after operation were recorded. The actuarial survival rate of the patients was calculated from the date of surgical resection of the colorectal cancers to the date of death or last follow-up.

RNA extraction and reverse transcription

Tissue blocks were sectioned into 7 micron slices for mRNA extraction. Preparation and mRNA extraction from these samples were performed as described previously [12]. PCR primers for *galectin* family members and controls are summarized in Table 1. A LightCycler 480[®] quantitative real-time PCR (Roche, Basel, Switzerland) was used to assess *galectin* family gene expression. All samples (unknown and standards) were run in triplicate and accompanied by a non-template control. Data analysis was performed as previously described [12]. A fold change of >2 was considered as high expression and a fold change expression of <1 was noted as low expression.

Tissue microarray

A tissue microarray (TMA) was constructed using a Model TMA Galileo CK3500 tissue Microarrayer (Integrated System Engineering Srl, Milano, Italy). Briefly, all representative tumour donor blocks were cut for haematoxylin & eosin staining to define the morphology and pathology of the representative regions. From those regions, 3 cylindrical core tissue specimens (diameter=0.6 mm) were arrayed into a recipient paraffin block (35 x 20mm²). Then, 4 μ m sections were cut from the TMAs and processed for immunohistochemistry.

Immunohistochemistry

Immunohistochemistry for galectin-1 and galectin-3 proteins was performed manually using a high pH EnVision™ FLEX Mini Kit (Dako, Michigan, USA). In brief, formalin-fixed, paraffin-embedded tissues were all sectioned to 4 µm. Then histological slides were deparaffinised and rehydrated in xylene and ethanol. Slides were heated in epitope retrieval buffer (Dako) for 15 minutes using a microwave at low pressure.

For galectin-1, slides were incubated with galectin-1 monoclonal antibody (sc-166618) (Santa Cruz biotechnology, California, U.S.A.) for 2 hours at room temperature. For galectin-3, a mouse monoclonal antibody was also recruited (sc-53127) (Santa Cruz biotechnology). After a series of initial optimisation steps, both primary antibodies for galectins-1 and 3 were diluted at 1:100 and incubated overnight at 4°C. The positive controls used were papillary thyroid carcinoma and colon adenocarcinoma tissue known to express these proteins. Omission of primary antibodies served as a negative control. Galectin-1 and galectin-3 cytoplasmic immunostaining intensity was examined and scored by the authors (NS & AKL) blinded to the RT-PCR results of the samples. The results were assessed semi-quantitatively and scored into 5 categories. Cases with no detectable immunostaining of carcinoma cells were scored zero. The remaining scoring was performed as follows- 1= weak (less than 10% positive); 2= moderate (10-29% positive), 3= strong (30% to 70% positive) and 4= extra strong (71% to 100%). Low protein expression was defined as scores of 0, 1 or 2 and high protein expression was defined as scores of 3 or 4.

Statistical analysis

Alterations in *galectin* mRNA and protein expressions, clinical, pathological, and follow up information were entered into a computerized database. Statistical analysis was

performed using the Statistical Package for Social Sciences for Windows (version 22.0, IBM SPSS Inc., New York, NY, USA). Significance level of the tests was taken at $p < 0.05$.

Results

Galectin family mRNA expressions in colorectal cancer tissues

Expression levels of *galectins* (Figure 1) and their predominance in colorectal cancer tissues is listed in table 2. Overall, over-expression of *galectins* were more common in colorectal cancer. The only exception was for *galectin-12*, which showed 72% (n=71/98) of samples with low expression in colorectal cancer.

Galectin mRNA expression and macroscopic features of the cancer

Compared to metachronous colorectal carcinomas, non-metachronous carcinomas (n=93) showed much higher mRNA expression of *galectin-4* (94% versus 60%, p=0.005) and *galectin-10* (84% versus 60%, p=0.012). *Galectin-2* mRNA expression was noted to be lower in patients with cancer perforation when compared to those without perforation (13% versus 60%, p=0.016). A similar trend was also noted with *galectin-7* mRNA expression in colorectal cancers with cancer perforation (60% versus 94%, p < 0.05).

Among colorectal carcinomas with associated polyposis (n=7/98) 57% (n=4/7) showed overexpression of *galectin-12* mRNA, whereas in carcinomas without associated polyposis only 19% (n=17/91) showed *galectin-12* high expression (p=0.053).

In rectal adenocarcinomas (n=20), *galectin-3* mRNA expression was noted to be evenly distributed (50% high versus 50% low), whereas 51% (n=40/78) of adenocarcinomas arising from colon showed high *galectin-3* expression and the remaining samples showed either low (24%) or normal (25%) levels of *galectin-3* mRNA expression (p=0.003).

Compared to proximal colon (caecum, ascending and transverse colon) (n=51), distal colorectal (descending colon, sigmoid colon and rectum) (n=47) cancers showed greater prevalence of *galectin-2* mRNA downregulation (73% versus 27%, p=0.039). There was a slight increase in *galectin-2* overexpression in proximal colon cancers when compared to cancers in distal colorectum (52% versus 48%, p=0.039).

Galectin-12 mRNA expression was often downregulated in colorectal adenocarcinomas of small size (maximum dimension ≤ 40 mm; n=48) when compared to larger carcinomas (> 40 mm; n=50) (83% versus 62%, $p=0.028$).

***Galectin* mRNA expression with microscopic features of the cancer**

Compared to conventional adenocarcinomas (n=84), mucinous adenocarcinomas (n=14) showed significantly more *galectin-2* mRNA overexpression (100% versus 68%, $p=0.007$). Also, *galectin-12* showed a similar trend in its expression pattern by 43% (n=6/14) of mucinous adenocarcinomas showing mRNA overexpression compared to conventional adenocarcinomas where only 18% (n=15/84) showed *galectin-12* overexpression ($p=0.027$). Also, *galectin-8* showed a trend of reducing its mRNA expression as the pathological grades of the carcinoma advanced (grade 1- 100%; grade 2- 98%; grade 3- 83%, $p= 0.011$).

Galectin 2, 8 and 12 mRNAs expression showed significance association with lympho-vascular invasion (LVI) in colorectal carcinomas. A slight predominance of *galectin-2* high expression was noted in colorectal adenocarcinomas without LVI (76%, n= 53/70) when compared to colorectal carcinomas with LVI (64%, n= 18/28) ($p=0.047$). Similarly, *galectin-8* overexpression was more prevalent in colorectal carcinomas without LVI when compared to those with LVI (99% versus 89%, $p=0.036$). Also, *galectin-12* showed an increased prevalence of low mRNA expression in colorectal carcinomas with LVI (86% versus 67%, $p=0.051$).

Pathological staging and *galectin* mRNA expressions

Significant differences in *galectins-2, 3, 7, 8 and 10* mRNA expression was noted amongst different pathological stages ($p<0.05$). *Galectin-3* mRNA was frequently overexpressed in early staged colorectal carcinomas when compared to advanced staged

cancers (58% in Stage I and 50% in Stage IV cancers, $p=0.010$). This trend was also noted with *galectin-7* and *galectin-2* mRNA expression. All colorectal adenocarcinomas of Stage I or Stage II showed *galectin-7* over expression ($n=50/50$) whereas 89% ($n=32/36$) and 67% ($n=8/12$) *galectin-7* overexpression was noted in carcinomas of Stage III or and Stage IV ($p=0.006$). Similarly, when compared to advanced staged colorectal cancers ($n=48$), *galectin-2* mRNA expression was higher in early staged cancers ($n=50$) of colorectum (82% versus 63%, $p=0.023$).

Analogous findings were observed for *galectins-2* and *7* in colorectal carcinomas with ($n=45$) and without ($n=53$) lymph node metastasis ($p < 0.05$). Overexpression of *galectin-2* mRNA was noted in 82% of colorectal carcinomas without lymph node metastasis and in only 62% of carcinomas without any lymph node metastasis ($p=0.015$). With *galectin-7* mRNA expression, this difference was more significant. Overexpression of *galectin-7* was noted in 100% of carcinomas without lymph node metastasis and in only 82% of carcinomas with lymph node metastases ($p=0.001$). Furthermore, a similar pattern of correlation was noted with *galectins-7, 8* and *10* mRNA expression and colorectal carcinomas with distant metastasis ($p < 0.05$).

***Galectin* mRNA expression with cancer recurrence and patient survival**

Recurrence or persistent cancer after operation was more often detected in patients with high levels of *galectins-4, 9* and *10*. *Galectins-1, 2, 3, 7, 8* and *12* showed increased prevalence of low mRNA expression in patients with cancer recurrence. None of these differences reached statistical significance ($p > 0.05$).

The survival rate of the patients with colorectal carcinoma was related to the pathological staging of the carcinoma ($p < 0.0001$) (Figure 2A). Patients with high level of *galectins-1, 3, 4* and *12* mRNA had slightly better survival rates than those with low

expression of these genes. Conversely, patients with high or normal levels of *galectin-2* mRNA expression showed slightly poorer survival rates when compared to patients with low mRNA levels ($p=0.078$).

Galectin-1 and galectin -3 proteins expression

Clinical and pathological correlation of galectin-3 protein expression was studied in 73 colorectal adenocarcinomas and 49 adenomas (Table 3)(Figure 3). In colorectal adenocarcinomas, approximately 95% ($n=69/73$) showed positive expression for galectin-3 protein, while only 73% ($36/49$) of colorectal adenomas has positive expression. Also, high grade staining (score 3 or 4) for galectin-3 protein was seen significantly more often in colorectal carcinomas when compared to adenomas (84% versus 31%, $p=0.0001$). A similar trend of expression changes was also noted with galectin-1 protein expression between colorectal adenocarcinomas ($n=55$) and adenomas ($n=52$). Colorectal adenocarcinomas showed positive expression for galectin-1 protein in approximately 62% of cases, whereas colorectal adenomas showed only 10% of positive galectin 1 expression. Additionally, 46% ($24/52$) of primary colorectal cancers showed correlation with expression levels of both proteins.

As with mRNA expression, colorectal adenocarcinoma patients having high levels of galectin-3 protein expression showed better prognosis when compared to those with no or low (score 0-2) galectin-3 protein expression (Figure 2B). However, this difference only showed marginal significance ($p=0.052$).

Sixty three per cent ($5/8$) of the colorectal adenocarcinomas with perforation showed significantly reduced galectin-3 protein expression, while 89% ($58/65$) of cancers without perforation showed increased levels of galectin-3 protein expression ($p=0.001$). With regards to pathological grades, the highest levels of expression for galectin-3 protein (score =4) were

found in 49% (n=28/57) of well/moderately differentiated colorectal adenocarcinoma and only 19% (n=3/16) of poorly differentiated colorectal carcinomas ($p=0.035$).

Galectin-3 protein showed some significant correlations with its expression pattern between primary adenocarcinomas and matched metastatic adenocarcinomas. All metastatic lymph node tissues with increased (score = 3 or 4) galectin-3 protein expression (n=12) showed a similar strength of expression with their primary colorectal carcinomas. Interestingly, 80% (n=8/10) of metastatic lymph nodes with an absence of galectin-3 protein showed over expression in their matched primary colorectal carcinomas ($p=0.043$).

In general, galectin-1 protein staining is weaker than galectin-3 in colorectal adenocarcinoma (Figure 4). With galectin-1 protein expression, only 40% (21/52) of primary colorectal adenocarcinomas showed positive expression while 50% (9/18) and 30% (8/26) of matched metastatic lymph nodes and distant metastatic tissues showed galectin-1 protein expression respectively. Also, amongst the available 27 cases of primary colorectal adenocarcinomas and metastatic adenocarcinoma in lymph nodes, approximately 59% of cases (n=16) showed direct correlation of galectin-1 protein expression between these tissues (No expression in 12 cases and positive expression in 4 cases). In addition, a concordant correlation of galectin-1 protein expression was noted in 48% (12/25) of primary colorectal adenocarcinoma and their matched metastatic adenocarcinoma in liver. Both of these correlations didn't reach statistical significance ($p > 0.05$).

The majority of patients with colorectal adenocarcinomas with persistent disease or cancer recurrence showed reduced levels of galectin-1 protein expression when compared to other patients (69% versus 38%; $p=0.047$). These associations were in concordance with galectin-1 mRNA expression.

Discussion

In colorectal carcinomas, galectins-1, 3, 4 and 8 have been reported to be deregulated in different stages of this carcinoma [8, 13-14]. In the current study, overexpression of *galectin* mRNA was often present, except for *galectin-12* which showed down regulation in 72% of the selected colorectal adenocarcinomas.

Galectins play a key role in cancer metastasis by virtue of their effects on cancer cell–matrix, cancer cell–cell and cancer-endothelial adhesion [8]. Laminin, carcinoembryonic antigen and lysosome-associated membrane glycoproteins are all potential galectin-3 cell surface ligands in galectin-3-mediated cell adhesion [15]. Galectins, especially galectin-3 influence cell growth and arrest by cross linking with epidermal growth factor receptor (EGFR) or transforming growth factor- β receptor (TGF β R) in colorectal carcinomas [16, 17]. In this study, *galectin-2* showed consistent correlation between its mRNA expression and cancer cell invasion and pathological staging. *Galectin-2* mRNA over expression was prevalent in colorectal carcinomas without lymph node or distant metastasis indicating the tumour initiative properties of galectin-2. It is worth noting that galectin-2 induces cell proliferation and can inhibit the release of pro-inflammatory cytokines [18]. Another study suggested that the significant down regulation of galectin-2 *in vivo* results in tumour suppression indicating the tumorigenic potential of galectin-2 [19]. These results also support the previous findings by Barrow *et al.* who showed increased serum levels of *galectin-2*, 4 and 8 mRNAs in patients with colon cancer metastasis, implicating it in cell adhesion effects in invasion [9]. Furthermore, once invasion and metastasis are initiated, tissue expression of galectins may not be required and it may be released into the serum as circulatory galectins [9].

Galectin-3 plays a key role in regulating colon cancer cells migration and distal localisation by mediating the K-Ras-Raf-Erk1/2 pathwa, as well as MUCI, a large

transmembrane mucin protein and a natural ligand of galectin-3 [20, 21]. The current study has found that increased levels of *galectin-3* mRNA expression were often noted with early stages colorectal carcinomas. This association can be related with the cell migration properties of *galectin-3* in the early stages of colorectal cancer development. Also, it has been suggested that the decreased galectin-3 may loosen the connection of cancer cells with the extracellular matrix to facilitate the invasive and metastatic properties of cancers [22].

A similar effect can be attributed to the expression and correlations between *galectin-4*, *7*, *8* and *10* mRNAs which showed slightly increased levels of their mRNA expression in colorectal carcinomas without any lymph node or distant metastasis. Analogous findings were also noted with early and late stage colorectal carcinomas. Additionally, these associations could be limited to the selected population of colorectal carcinomas as not all colon cancers follow the galectin mediated cell proliferation and metastasis. This theory is further strengthened by the fact that a significant population (30-80%) of highly advanced colorectal carcinomas exhibited *galectin* overexpression. For example, despite the reduced trend of *galectin-8* overexpression in colorectal carcinomas with different pathological grades (from 100% to 80% in grade 1 to 3 carcinomas), 83% of grade 3 colorectal carcinomas still exhibited *galectin-3* overexpression when compared to non-cancer colorectal tissues. It is also possible that the increased *galectin* expression in early stage carcinomas may be related to the acquisition of mutations or gene network alterations that lead to the development of invasive and metastatic potential in primary cancer cells.

In the large intestine, the clinical, pathological and molecular features in synchronous and metachronous cancers are different from conventional cancers [23, 24]. The current study reported for the first time the association of *galectin* mRNA expression with metachronous and non-metachronous carcinomas in colorectum. Higher prevalence of *galectin-4* mRNA and *galactin-10* mRNA overexpression was seen in non-metachronous adenocarcinomas

when compared to metachronous cancers. There is also significant association of *galectin-2* and *10* expressions in colorectal adenocarcinomas from different histological subtypes.

Increased prevalence of high mRNA expression of these *galectins* in mucinous adenocarcinomas when compared to conventional adenocarcinomas implies the significance of *galectin-2* and *10* in predicting biologically aggressive colorectal carcinomas.

Differential expression patterns for several genes in anatomical sites of the large intestine have been reported [12]. In this study, the site dependent expression changes in *galectin-2* and *3* mRNAs imply a potentially differential role of these *galectins* based on the site of origin of tumours. Furthermore, these differences in *galectin* expression in different sites of colorectum may have implications regarding specifically targeted therapeutic regimens in the future.

To validate the mRNA expression and to underpin the clinicopathological correlation, this study has examined galectin-1 and galectin 3 proteins in selected colorectal adenocarcinomas and adenomas. Galectin-1 protein has been reported to be over expressed in colorectal adenocarcinomas when compared to normal colonic mucosa and adenomas, as well as in cancers with increase metastatic potential [25, 26]. Similar findings were also noted with galectin-1 protein in this study. Compared to primary colorectal adenocarcinomas, the metastatic lymph node tissues showed increased expression of galectin-1 protein and high galectin-1 protein expression between primary and metastatic lymph nodes in 59% of cases suggests the significance of this galectin in colorectal cancer metastasis. These results, along with data in literature imply that galectin-1 can act as a potential target for therapies to combat cancer metastasis. This is related to the fact that all evidence indicates that galectin has a role in transitioning cancer from its local site to widespread invasiveness.

Galectin 3 protein is commonly overexpressed in colorectal cancer tissues [27]. The results of the current study on galectin-3 protein concurred with the previous findings, as

95% of the primary colorectal adenocarcinomas showed increased levels of galectin-3 protein compared to adenomas (73%). Galectin-3 protein was also expressed at a higher level in advanced colorectal cancer tissues, with metastatic carcinomas expressing higher levels of galectin-3 protein than the primary tumours from which they arise [27]. In contrast, we found that galectin-3 protein expression was significantly less in colorectal carcinomas with poor differentiation or perforation. Similarly, high galectin-3 protein and mRNA expression was noted in patients with better prognosis. However, this contradictory result supports the finding in one previous study, which reported comparatively low galectin-3 protein expression in pathologically aggressive colorectal carcinomas [25]. These results suggest that galectin-3 protein shows altered behaviour in different stages of colorectal carcinogenesis with high activity of galectin-3 protein in initial phases of tumour progression and during the advanced stages it may be lost in favour of other galectins or molecular pathways.

Studies have reported that galectin-3 expression can predict prognosis of colorectal cancer patients [27]. Based on these studies, disease free survival for colorectal cancer patients was shorter when they expressed high galectin-3 protein or mRNA levels in their primary colorectal carcinoma. Conversely, our study has noted that colorectal cancer patient survival was better when they had a high galectin-3 protein in their primary cancer tissues. We hypothesize that these contradictory findings could be attributed to changes in cancer dependent subcellular localization. Normal colonic mucosa shows strong nuclear expression of galectin-3 protein and its expression is noted to be high in advanced carcinomas when they exhibit a nuclear-cytoplasmic trans-localization in colon cancer cells [8]. This cytoplasmic galectin-3 is known to be an apoptosis inhibitor and this change in localization may contribute to cell survival in colorectal cancers [28]. Galectin-3's switch from nucleus to cytoplasm is mediated by serine 6 phosphorylation in its amino acid sequence, which in turn regulates the

anti-apoptotic activity of galectin-3 [29]. On the other hand, nuclear localization of galectin-3 is associated with normal cell proliferation and acts as a positive regulator of proliferation [30]. The current study has shown galectin-3 protein staining in both nuclear and cytoplasmic locations. Our hypothesis that high galectin-3 expression can be indicative of good prognosis are supported by the findings of Shimamura et al. in which pancreatic ductal carcinoma patients with high galectin-3 protein expression showed better prognosis [31]. Thus, the change in expression pattern and localization of galectin-3 expression might depend on different cancer types and might have a different function in various stages in carcinogenesis by controlling the cell proliferation via regulation of cellular apoptosis.

The current study reported many novel correlations between the deregulation of galectin family genes and various clinicopathological features in a large series of colorectal adenocarcinomas. Similar to mRNA expression changes, altered expression pattern was noticed for both galectin-1 and galectin-3 proteins with high activity of these proteins in initial phases of tumour progression. Also, these altered expression patterns of galectin genes suggest the multi-functional role of these molecules in the regulation of colorectal cancer development, progression and metastasis. Furthermore, detailed knowledge of the expression and roles of these galectins in the progression of human colorectal carcinomas at tissue level along with future studies on their ligands can be used as potential therapeutic targets for colorectal cancer treatment.

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Figure Legends

Figure 1

Expression profiling of galectin family mRNA in colorectal carcinomas: Altered expression patterns were noted for different galectin family genes at the mRNA level. Y-axis of each graph represents inverse ratio of mean CT value (RR) of each galectin against the control gene GAPDH.

Figure 2

The relationship between patients' survival and Galectin-3 protein expression levels in colorectal carcinoma: A- The survival rate of the patients with colorectal carcinoma was related to the pathological staging of the carcinoma ($p < 0.0001$). B- Patients with high galectin-3 protein expression levels (immuno score 2^+ and 3^+) in their colorectal cancer tissue had slightly better survival rates than patients with no or low galectin-3 protein (immuno score 0 and 1^+) ($p=0.052$). These results were consistent with galectin-3 mRNA expression levels (image not shown).

Figure 3

Galectin-3 protein expression:

Colorectal adenocarcinoma revealing strong cytoplasmic and nuclear staining (x 10)

Figure 4

Galectin-1 protein expression:

Metastatic colorectal adenocarcinoma revealing weak cytoplasmic and nuclear staining (x 16)

Conflict of interest:

There are no financial and personal relationships with other people or organizations that could inappropriately influence (bias) the work.

Table 1**PCR primers forward and reverse for galectin family members used in this study**

Galectin member	product size (base pairs)		Sequence
<hr/>			
Galectin-1	Product 84	Forward	TGCAACAGCAAGGACGGC
		Reverse	CACCTCTGCAACACTTCCA
Galectin-2	Product 94	Forward	GATGGCACTGATGGCTTTG
		Reverse	AGACAATGGTGGATTTCGCT
Galectin-3	Product 108	Forward	CAGAATTGCTTTAGATTTCCTAA
		Reverse	TTATCCAGCTTTGTATTGCAA
Galectin-4	Product 144	Forward	CGAGGAGAAGAAGATCACCC
		Reverse	CTCTGGAAGGCCGAGAGG
Galectin-7	Product 141	Forward	CAGCAAGGAGCAAGGCTC
		Reverse	AAGTGGTGGTACTGGGCG
Galectin-8	Product 95	Forward	CTTAGGCTGCCATTTCGCT
		Reverse	AAGCTTTTGGCATTTCGA
Galectin-9	Product 91	Forward	CTTTCATCACCACTTCTG
		Reverse	ATGTGGAACCTCTGAGCACTG
Galectin-10	Product 114	Forward	AGTGTGCTTTGGTCGTCGT
		Reverse	ATGCTCAGTTCAAATTCTTGG
Galectin-12	Product 111	Forward	TGTGAGCCTGAGGGACCA
		Reverse	GCTGAGATCAGTTTCTTCTGC
GAPDH	Product 87	Forward	TGCACCACCAACTGCTTAGC
		Reverse	GGCATGGACTGTGGTCATGAG
18S Ribosome	Product 71	Forward	CTTAGAGGGACAAGTGGCG
		Reverse	GGACATCTAAGGGCATCACA

Table 2: Altered mRNA expression levels of galectin family genes in colorectal adenocarcinoma.

Galectin family	High expression	Low expression	Normal expression
Galectin-1	40 (40.8%)	56 (57.1%)	2 (2.0%)
Galectin-2	71 (72.4%)	15 (15.3%)	12 (12.2%)
Galectin-3	50 (51.0%)	29 (29.6%)	19 (19.4%)
Galectin-4	90 (91.8%)	2 (2.0%)	6 (6.1%)
Galectin-7	90 (91.8%)	7 (7.1%)	1 (1.0%)
Galectin-8	94 (95.9%)	0 (0.0%)	4 (4.1%)
Galectin-9	88 (89.8%)	3 (3.1%)	7 (7.1%)
Galectin-10	81 (82.7%)	10 (10.2%)	7 (7.1%)
Galectin-12	21 (21.4%)	71 (72.4%)	6 (6.1%)

Table 3
The relationship between clinicopathological features and Galectin-3 protein expression in colorectal adenocarcinoma

Features	Total Number	Low Expression (0, 1+)	High Expression (2+/3+)	p-value
Gender				
Male	36	1 (3%)	35 (97%)	0.317
Female	37	3 (8%)	34 (92%)	
Age				
Age ≤ 60	24	1 (4%)	23 (96%)	0.601
Age > 60	49	3 (6%)	46 (94%)	
Site				
Colon	54	3 (5%)	51 (95%)	0.724
Rectum	19	1 (5%)	18 (95%)	
Grade				
Well	6	1 (2%)	5 (98%)	0.045
Moderate	51	3 (6%)	48 (94%)	
Poor	16	0 (0%)	16 (100%)	
Histological type				
Conventional	63	4 (6%)	59 (94%)	0.715
Mucinous	10	0 (0%)	10 (100%)	
Size				
Size ≤ 50 mm	32	2 (6%)	30 (94%)	0.075
Size > 50 mm	41	2 (5%)	39 (95%)	
Lymphovascular permeation				
Positive	39	1 (3%)	38 (97%)	0.257
Negative	34	3 (9%)	31 (91%)	
Peri-neural invasion				
Positive	18	2 (11%)	16 (88%)	0.253
Negative	55	2 (4%)	53 (96%)	
Perforation				
Positive	8	1 (63%)	7 (27%)	0.001
Negative	65	3 (11%)	62 (89%)	
T-stage				
I&II	4	0 (0%)	4 (100%)	0.620
III&IV	69	4 (6%)	65 (94%)	
Lymph node				
Positive	48	3 (6%)	45 (94%)	0.576
Negative	25	1 (4%)	24 (96%)	
Distant metastases				
Positive	25	2 (8%)	23 (92%)	0.184
Negative	48	2 (4%)	46 (96%)	
TNM stage				
I&II	22	1 (5%)	21 (95%)	0.651
III&IV	51	3 (6%)	48 (94%)	
Recurrence/ Persistent cancer				
Yes	46	4 (7%)	42 (93%)	0.050
No	27	0 (0%)	27 (100%)	