The mammalian galectins are a family of 15 proteins characterized by binding affinity for β-galactose-containing glycoconjugates and at least one conserved carbohydrate recognition domain (CRD) [1]. From the viewpoints of protein architecture, they have been classified into prototype galectins (galectin-1, -2, -5, -7, -10, -11, -13, -14, and -15), which exist as monomers or non-covalent homodimers of a CRD, chimera-type galectin (galectin-3) composed of a non-lectin domain connected to a CRD, and tandem-repeat-type galectins (galectin-4, -6, -8, -9, and -12) consisting of two different CRDs in a single polypeptide chain. However, this nomenclature does not accurately reflect the evolutionary relationship between the members of this family. Indeed, the mono-CRD galectins can be split into two subgroups, the F4 and F3 types, which are evolutionarily quite distinct from each other. The chimeric galectin-3 is a member of the F3 subgroup [2]. Although most galectins bind preferentially to glycoproteins containing the ubiquitous disaccharide N-acetyl-lactosamine, individual galectins interact with specific spatially available glycan termini of cellular glycoconjugates, leading to well-defined sugar–sugar interactions. Moreover, numerous functions unrelated to lectin activity have been revealed, in particular for galectin-3 [3]. Such multiplicity and diversity of interactions make galectins involved in various biological processes. Galectins play different roles in cell–cell and cell–matrix adhesion, as well as in internal processes [4]. For example, galectin-1 and -3 have been identified as Ras escort/regulator proteins [5] and constitute part of an interacting dynamic network of many factors involved in the splicing and transport of mRNA [4]. Moreover, galectins seem to be master regulators of immune cell homeostasis and inflammation, either by regulating cell survival and signalling, influencing chemotaxis, or interfering with cytokine secretion [6,7]. Such biological roles of galectins are dependent on cellular localization (nuclear, cytoplasmic or extracellular) but also on the cell type involved. Galectin-1 null mutant [8] and galectin-3 null mutant mice [9] are, however, viable and have no overt abnormalities, indicating either that these galectins are not required for survival under conventional animal house conditions or that functional redundancy operates between the family members.

Galectins have been detected in many cell and tissue types, with specific patterns of expression, in normal as well as in pathological situations; these observations render them potentially interesting biomarkers.
for diagnostic and prognostic purposes in daily clinical practice. Furthermore, the development of targeted therapies could offer interesting potentialities for therapeutic modalities involving galectins. With this regard, we reviewed the numerous reports illustrating the role of galectins in the digestive tract. The galectin expression in normal tissue is compared with neoplastic as well as inflammatory conditions in the digestive tract, with links to their putative biological role. We also highlighted the potential clinical and therapeutic tools which could emerge from the reported data.

**Galectin expression in the normal digestive tract**

As illustrated in Table 1, the mammalian digestive tract is particularly rich in galectins since nine subtypes (galectin-1, -2, -3, -4, -6, -7, -8, -9, and -15) have been identified. Among these, galectin-2, -6, -7, -9, and -15 have been detected only in non-human species. With regard to galectin-5, -10, -11, -12, -13, and -14, neither the proteins nor their mRNA has been demonstrated in the digestive tract. In humans, galectin-1 and -8 are found in both the nucleus and the cytoplasm of colonic epithelial cells, as well as in stromal cells [10–12]. In murine intestine, galectin-3 was detected in duodenal and colonic epithelium by means of immunohistochemistry, which demonstrated expression in the cytoplasm and nucleus and at the cell surface [13]. A similar pattern was observed in the epithelium of human stomach, ileum, and colon, with stromal expression in the colon only [10,14–19]. One group observed galectin-3 expression in the oesophagus [20]. During development and in the adult, galectin-4 is expressed only in the epithelium of the gastrointestinal tract, from the tongue to the colon [21,22]. Galectin-4 mRNA has been detected in the small and large intestine and, to a lesser degree, in the stomach of rats [23] and mice [22]. Immunohistochemistry revealed the presence of this protein in oesophageal squamous epithelium [24] and small intestinal epithelium in pigs [25], and colonic epithelium in humans [21] and mice [26].

**The diseased colorectum**

**Colorectal cancer**

As summarized in Table 2 and detailed below, the most important data on galectin expression in the colorectum are the observations that overexpression of galectin-1 is associated with dysplastic transformation and neoplastic progression [10,11,27]; that high levels of galectin-3 correlate with neoplastic progression [11,15–17,27–29]; and that galectin-8 expression decreases markedly during tumourigenesis in the human colon [12,27].

**Galectin-1**

Overexpression of galectin-1 is associated with dysplastic transformation [10,11] and neoplastic progression [10,11,27] in colorectal cancer. A prognostic value associated with the expression levels of galectin-1 has been observed in Dukes’ A and B tumours, independent of Dukes’ stage [27]. It must be mentioned, however, that this overexpression is mainly detected in stromal cells. Galectin-1 overexpression could also be used as a therapeutic indicator, since a recent gene expression profile study revealed that responders to pre-operative radiotherapy for rectal cancer show higher galectin-1 expression than non-responders [30].

**Galectin-3**

In colon cancer, increased levels of galectin-3 correlate with neoplastic progression [11,15–17,27–29]. Six additional studies included adenomas with partial results that do not allow a global conclusion, essentially due to lack of information on the degree of dysplasia [10,11,14,15,28,29]. Moreover, marked changes in the subcellular location of galectin-3 occur during colon carcinoma progression, with loss of nuclear galectin-3 in colon cancer cells [14]. Sanjuan et al demonstrated that nuclear galectin-3 expression is down-regulated in the initial stages of neoplastic progression, whereas cytoplasmic expression increases in the later phases of tumour progression [10]. The mechanism allowing this altered cytolocation of galectin-3 remains unclear. Interestingly, studies with murine and

<table>
<thead>
<tr>
<th>Galectin</th>
<th>Oesophagus</th>
<th>Stomach</th>
<th>Small intestine</th>
<th>Colon</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Human [20]</td>
<td>Rat [85]</td>
<td>Rat [85]</td>
<td>Human [10,11], mouse [42,84]</td>
</tr>
<tr>
<td>4</td>
<td>Rat [86], mouse [86]</td>
<td>(Human) [86]</td>
<td>(Rat, mouse) [86]</td>
<td>Human [21], rat [23], mouse [22,26]</td>
</tr>
<tr>
<td>5</td>
<td>Rat [87], mouse [87]</td>
<td>Sheep [88]</td>
<td>Mouse [22]</td>
<td>Mouse [22]</td>
</tr>
<tr>
<td>6</td>
<td>(Rat, mouse) [86]</td>
<td>—</td>
<td>—</td>
<td>— (Rat, mouse) [86]</td>
</tr>
<tr>
<td>7</td>
<td>Sheep [88]</td>
<td>—</td>
<td>—</td>
<td>Human [12]</td>
</tr>
</tbody>
</table>

Table 1. Location of galectins in the mammalian digestive tract, independent of methods of detection (immunohistochemistry, western blot, reverse transcriptase-polymerase chain reaction, in situ hybridization). Negative results (–) in Table 1 must be interpreted with caution, given the limited number of studies.
### Table 2. Altered galectin expression in tissue samples from patients with diseases of the digestive tract and potential clinical implications

<table>
<thead>
<tr>
<th>Disease</th>
<th>Galectin</th>
<th>Alteration</th>
<th>Technique</th>
<th>Clinical implication</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorectal adenoma and cancer: galectin-1 overexpression as a marker of dysplastic transformation and neoplastic progression</td>
<td>1</td>
<td>Increased expression</td>
<td>Semi-quantitative IH (non-commercial rabbit PAb), WB</td>
<td>—</td>
<td>10</td>
</tr>
<tr>
<td>Colorectal adenoma and cancer</td>
<td>1</td>
<td>Increased expression</td>
<td>Semi-quantitative IH (non-commercial rat PAb)</td>
<td>—</td>
<td>11</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>1</td>
<td>Increased expression</td>
<td>Quantitative computer-assisted IH (non-commercial rabbit pAb)</td>
<td>Prognostic value in Dukes’ A and B tumours</td>
<td>27</td>
</tr>
<tr>
<td>Colorectal cancer; galectin-3 overexpression as a marker of neoplastic progression with prognostic value</td>
<td>3</td>
<td>Increased expression</td>
<td>FC (9H3.2 mouse MAb)</td>
<td>Strong cytoplasmic expression in stage II and III carcinomas associated with worse prognosis</td>
<td>29</td>
</tr>
<tr>
<td>Colorectal adenoma</td>
<td>3</td>
<td>Increased nuclear expression; decreased cytoplasmic expression only in adenomas</td>
<td>Semi-quantitative IH (M3/38 rat MAb), IB, NB</td>
<td>Related to CEA serum level and clinical stage</td>
<td>14</td>
</tr>
<tr>
<td>Colorectal adenoma and cancer</td>
<td>3</td>
<td>Decreased expression in adenoma; increased expression in cancer</td>
<td>Semi-quantitative IH (non-commercial rabbit PAb), IB</td>
<td>Related to Dukes’ stage and decreased long-time survival</td>
<td>28</td>
</tr>
<tr>
<td>Colorectal adenoma and cancer</td>
<td>3</td>
<td>Increased expression</td>
<td>Semi-quantitative IH (TIB166 rat MAb)</td>
<td>Related to clinical stage and prognosis</td>
<td>17</td>
</tr>
<tr>
<td>Colorectal adenoma and cancer</td>
<td>3</td>
<td>Increased expression</td>
<td>Semi-quantitative IH (TIB166 rat MAb)</td>
<td>Prognostic value in Dukes’ A and B tumours</td>
<td>27</td>
</tr>
<tr>
<td>Rectal cancer</td>
<td>3</td>
<td>Increased expression</td>
<td>DNA microarray</td>
<td>Associated with pre-operative radiotherapy response</td>
<td>30</td>
</tr>
<tr>
<td>Colorectal cancer; galectin-4 overexpression with prognostic value to validate</td>
<td>4</td>
<td>Increased expression</td>
<td>Quantitative computer-assisted IH (non-commercial rabbit PAb)</td>
<td>Prognostic value in Dukes’ A and B tumours</td>
<td>27</td>
</tr>
<tr>
<td>Colorectal cancer; galectin-8 down-expression with prognostic value</td>
<td>8</td>
<td>Decreased expression and exclusion from the nucleus in cancer</td>
<td>Quantitative computer-assisted IH (non-commercial rabbit PAb)</td>
<td>Related to metastatic potential</td>
<td>12</td>
</tr>
<tr>
<td>Colorectal adenoma and cancer</td>
<td>8</td>
<td>Decreased expression</td>
<td>Quantitative computer-assisted IH (non-commercial rabbit PAb)</td>
<td>Prognostic value in Dukes’ C and D tumours</td>
<td>27</td>
</tr>
<tr>
<td>Other disease locations: few data with clinical implications to clarify</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oesophageal squamous cell carcinoma</td>
<td>3</td>
<td>Increased expression in nuclei</td>
<td>IH (9C4 mouse MAb)</td>
<td>Inverse correlation with differentiation and vascular invasion</td>
<td>20</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>3</td>
<td>Increased expression</td>
<td>Semi-quantitative IH (Mac-2 mouse MAb)</td>
<td>Nuclear positivity stronger in diffuse type than in intestinal type</td>
<td>18</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>3</td>
<td>Increased expression</td>
<td>Semi-quantitative IH (B2C10 mouse MAb)</td>
<td>Related to clinical stage</td>
<td>19</td>
</tr>
<tr>
<td>Barrett’s oesophagus</td>
<td>4</td>
<td>Increased expression</td>
<td>SAGE, RT-PCR, IB</td>
<td>—</td>
<td>49</td>
</tr>
<tr>
<td>Crohn’s disease</td>
<td>3</td>
<td>Decreased expression in ileal and colonic epithelium</td>
<td>RT-PCR, IH (Mac-2 mouse MAb)</td>
<td>—</td>
<td>45</td>
</tr>
</tbody>
</table>

*FC = flow cytometry; IB = immunoblot; IH = immunohistochemistry; MAb = monoclonal antibody; NB = northern blot; PAb = polyclonal antibody; RT-PCR = reverse transcriptase-polymerase chain reaction; SAGE = serial analysis of gene expression; SB = slot blot; WB = western blot.*
human fibroblasts revealed that both phosphorylated and unphosphorylated galectin-3 are present in the nucleus, but only the phosphorylated form is cytoplasmic [31]. Phosphorylation reduces the binding of galectin-3 to laminin and mucin by more than 85% [32] and is required for its anti-apoptotic and anti-anoikis activities [33], which are thought to be critical for anchorage-independent cell survival in the circulation during dissemination; therefore, altered galectin-3 cytolocation with an increase in cytoplasmic and phosphorylated galectin-3 might render metastatic potential to neoplastic cells.

All authors agree that increased galectin-3 expression in colorectal cancer is associated with a poor prognosis [10,16,17,27,28]. More controversial data exist on the relationship between galectin-3 expression and metastatic potential of colorectal cancers; reduced expression of galectin-3 was shown to be associated with metastatic capabilities of the primary tumour [34], whereas others observed an inverse relationship [17]. These latter data are consistent with the observation that up-regulation of galectin-3 in colon cancer cells by stable transfection results in an increase in spontaneous metastasis and liver colonization, while down-regulation by antisense methodology significantly reduces metastasis [35].

Interestingly, galectin-3 upregulates MUC2 mucin transcription through AP-1 activation [36]. MUC2 is expressed strongly in mucinous carcinomas [37] and patients with mucinous colorectal carcinomas characteristically have advanced disease at presentation [38].

Additionally, assessing galectin levels in serum might be an interesting clinical tool. Indeed, compared with healthy individuals, galectin-3 serum levels in patients with colorectal cancer are significantly greater, with maximal concentrations found in patients with metastatic diseases [39]. However, the source of increased serum galectin-3 remains unclear and may involve tumour cells as well as inflammatory cells expressing galectin-3 showing an immune reaction to the tumour load [39]. Additional research is warranted to determine the clinical value of circulating galectin levels in patients with early-stage cancer as a predictor of tumour spread. This should investigate whether a decrease in serum galectin-3 parallels a disease-free state and whether increasing galectin-3 serum levels in patients with early-stage cancer as a predictor to determine the clinical value of circulating galectin in metastatic diseases [39].

We demonstrated that poor prognosis is associated with increased percentages of colon carcinoma cells expressing galectin-4 in Dukes’ A and B tumours [27]. In our series, the combination of galectin-4 and galectin-1 expression was shown to provide more useful prognostic information than galectin-3 and Dukes’ staging [27]. In T84 human colon adenocarcinoma cells, galectin-4 and galectin-3 are distributed to specific lamellipodia domains, suggesting their cooperation in cell–substrate interaction [40]. These data suggest that an increase of galectin-4 in colon carcinoma might facilitate cell migration from the primary tumour to the site of metastasis. Formal proof for this hypothesis, however, is lacking.

**Inflammatory bowel disease (IBD)**

While several galectin ligands involved in inflammatory responses are widely distributed in the gut (Table 3), there is a lack of data on galectins in the inflammatory digestive tract. Galectin-1 is able to down-regulate the immune response, whereas galectin-3 and -4 behave as amplifiers of the inflammatory cascade.

**Table 3. Galectins and their ligands in the human digestive tract**

<table>
<thead>
<tr>
<th>Galectin</th>
<th>Ligands</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fibronectin [89], laminin [89], Mac-2 binding protein [90], α5β1-integrin [66], actin [89], CD7 [89], CD43 [89], CD45 [89], CD2 [89], CD3 [89], CD4 [89], CD107a (LAMP-1) [89], CD107b (LAMP-2) [91], gastrointestinal mucin [89], H-Ras [53], K-Ras [53]</td>
</tr>
<tr>
<td>3</td>
<td>Fibronectin [52], laminin [52,92], elastin [93], collagen [92], hersin (DMBT-1) [94], Mac-2 binding protein [90], β1-integrin (CD29) [55], C44A [95], CD95 (Fas) [96], CD7 [55], CD11b [91], CD98 [91], CD107a (LAMP-1) [97], CD107b (LAMP-2) [97], CEA [98], MUC2 mucin [99], TCR complex [100], Mac-3 [91], NG2 proteoglycan [101], β-catenin [63], AGE products [102], avin [64], H-Ras [67], K-Ras [67], synexin [73]</td>
</tr>
<tr>
<td>4</td>
<td>Sulphatide [103]</td>
</tr>
<tr>
<td>8</td>
<td>Fibronectin [41], α3-integrin [41], CD29 (β1-integrin) [41]</td>
</tr>
</tbody>
</table>

**Galectin-4**

Fibronectin [89], laminin [89], Mac-2 binding protein [90], α5β1-integrin [66], actin [89], CD7 [89], CD43 [89], CD45 [89], CD2 [89], CD3 [89], CD4 [89], CD107a (LAMP-1) [89], CD107b (LAMP-2) [91], gastrointestinal mucin [89], H-Ras [53], K-Ras [53]
Administration of high concentrations of galectin-1 creates a micro-environment with high IL-10 levels [43,44] and thus may be beneficial in the treatment of inflammatory digestive diseases. However, further studies are needed to address the interesting possibilities with this immunosuppressive galectin, considering the timing and complexity of the different processes playing a role in human IBD.

Down-regulation of galectin-3 in intestinal epithelium in Crohn’s disease has been reported, possibly as a consequence of enhanced tumour necrosis factor-alpha (TNF-α) production by inflammatory cells [45]. Since soluble galectin-3 is a strong lamina propria fibroblast-stimulating factor, it may be speculated that this molecule could be involved in the induction of fibrosis in Crohn’s disease [46].

Galectin-4 might also be involved in the pathogenesis of IBD, since it specifically stimulates IL-6 production under inflammatory conditions by interacting with the immunological synapse generated on colonic lamina propria CD4+ T-cells. This leads to an exacerbation of chronic intestinal inflammation and a delay in the recovery from acute inflammation [26]. This galectin-4-mediated IL-6 production was major histocompatibility complex class II-independent [26]. It was postulated that the production of galectin-4 by epithelium is up-regulated during intestinal inflammation, but this hypothesis could not be confirmed [26]. However, the presence of elongated epithelial crypts indicating an increased number of epithelial cells per colon is a common feature in chronic colitis [47]. Therefore, the increased epithelial cell mass may result in an increased total amount of circulating galectin-4.

The diseased upper digestive tract

In contrast to the colorectum, only a few studies are dedicated to the involvement of galectins in diseases of the upper digestive tract. In oesophageal squamous cell carcinoma, high nuclear galectin-3 expression has been shown to correlate inversely with vascular invasion and histological differentiation, whereas cytoplasmic expression had no impact on clinicopathological factors [20]. Neither nuclear nor cytoplasmic galectin-3 expression, however, was a prognostic indicator [20].

Increased galectin-3 expression has been observed in gastric adenocarcinoma, suggesting that it could be a useful marker, with respect to tumour progression and presence of lymph node metastasis, especially in poorly differentiated adenocarcinoma [19]. Nuclear galectin-3 reactivity is significantly stronger in diffuse-type cancers than in intestinal-type tumours, and this reactivity does not correlate with the incidence of Ki-67-positive tumour cells [18].

As for colorectal cancer, galectin-3 serum levels are increased in patients with gastric cancer, with maximal concentrations found in patients with metastatic diseases [39].

With regard to galectin-4, one report mentions preferential up-regulation of galectin-4 in gastric cancer cell lines prone to peritoneal dissemination [48]. Additionally, a recent study comparing the transcripts of Barrett’s oesophagus and normal squamous oesophageal epithelium revealed an up-regulation of galectin-4 in metaplastic epithelium of Barrett’s oesophagus [49].

Galectin ligands and putative functions in inflammatory and neoplastic conditions in the digestive tract

Table 3 summarizes the galectin ligands relevant for the human digestive tract. The large majority are glycoproteins located in the extracellular matrix or at the cell surface, whereas others are cytoplasmic or nuclear proteins.

It becomes more and more evident that galectins are important inflammatory mediators and may function as pro- or anti-inflammatory actors [6,7]. Many CD antigens are ligands for galectins, especially for galectin-3 and -1. This leads to increased leucocyte recruitment, transmigration, and adhesion to extracellular matrix, and to bacterial phagocytosis with galectins acting as opsonins. As opposed to the chemotactic activity observed with galectin-9 for eosinophils and galectin-3 for macrophages, galectin-1 has been shown to inhibit inflammatory cell migration [50].

A large body of evidence has highlighted the importance of interactions between cancer cells and carbohydrate residues during cancer progression [51]. Galectin involvement in digestive cancers is well documented, but despite the specific binding sites listed in Table 3, there are still controversies in the overall understanding of the role of galectins in cell adhesion [49].

Parts of the pro-tumoural activities of certain galectins are related to their anti-apoptotic role. Loss of cell–cell or cell–matrix interactions can lead to apoptotic cell death (anoiokis), and galectin-3 is clearly associated with anoiokis resistance [52]. The binding of galectin-1 to oncogenic H-Ras is an example of a salient cytoplasmic interplay relevant for tumour biology [53,54]. The same holds true for the way that galectins gain entry to the apoptosis signalling cascade affecting proteins or to pre-mRNA splicing [4].

Another pro-tumoural galectin activity is linked to their ability to down-regulate the immune attack. Extracellular galectin-1, -3, and -9 induce CD8+ T-cell apoptosis and contribute to the immune escape of cancer cells [55,56].

Part of the regulation of galectin functions is due to ligand affinity modulation, eg phosphorylation of galectins significantly altering the interaction with ligands [32]. The process by which phosphorylation acts as an ‘on–off’ switch for protein–carbohydrate interactions is unknown, but it has important implications for understanding the biological functions of galectins.
The galectin phosphorylation status also influences their subcellular location, leading to different functions. Recently, emerging results are unravelling the different levels of affinity regulation, acting in a dynamic manner and combining substitutions (e.g. core fucosylation) with spatial arrangements of presentation [57]. Evidently, the enzymatic modification of glycan structures can be linked to changes in (traffic) signal display along highways, with a strong bearing on galectin-binding properties. In consequence, cell adhesion and other activities of matricellular effectors such as galectin-1, -3, and -8 hereby become swiftly subject to regulation [58]. Thus, the so far phenotypically mapped changes in glycosylation gain a functional dimension for cell sociology via lectin presence.

As detailed below, it is becoming clear that intracellular galectins are involved in several signalling pathways that regulate cell growth or apoptosis in cancer cells. In Figures 1, 2, and 3, the roles of galectin-1, -3, and -7 in colon cancer are illustrated in blue, green and pink, respectively. Symbolic representation of signal-transduction controls is as proposed by Pirson et al [59].

**Wnt signalling pathway**

This pathway regulates a variety of processes such as cell proliferation, differentiation, migration, as well as survival/apoptosis, and is frequently deregulated in human tumours. At a resting state (absence of Wnt signalling), glycogen synthase kinase-3β (GSK-3β) as well as casein kinase 1α (CK1α) phosphorylate β-catenin, marking it for ubiquitination and degradation by the proteasome (Figure 1). Phosphorylation of β-catenin occurs in a complex that includes axin, APC (adenomatous polyposis coli), GSK-3β, and CK1α [60–62]. The presence of Wnt signalling disrupts the complex, resulting in the accumulation of free β-catenin and its translocation to the nucleus (Figure 1). Binding of nuclear β-catenin to TCF/LEF proteins leads to the formation of a multimeric complex containing transcriptional co-activators, such as CBP/p300, that induces the transcription of the Wnt target genes. Cyclin D1, MMP7, CD44, and c-myc are among the genes activated and relevant to tumour progression (Figure 1) [60–62]. In colorectal cancer, mutations of several proteins in this pathway (APC, axin or β-catenin itself) have been described. Such mutations can prevent the degradation of β-catenin, leading to an inappropriate induction of genes regulated by TCF/LEF. For example, the frequency of mutation of APC in sporadic colorectal cancer is approximately 80%, while that of β-catenin is close to 10% [61]. Interestingly, like β-catenin, galectin-3 can be phosphorylated by CK1α and GSK-3β [63,64]. Galectin-3 also interacts with both axin and β-catenin (Figure 1) [63,64], and it is tempting to speculate that galectin-3 could be a member of the cytosolic complex that regulates β-catenin level and activity. Moreover, in different cell lines including HT29 and HCT116 colon cancer cells, galectin-3 binds to the nuclear complex formed by β-catenin and TCF/LEF: galectin-3 co-localizes with β-catenin in the nucleus and stimulation of the expression of c-myc and cyclin D1 genes by β-catenin relies, at least partly, on the presence of galectin-3 (Figure 1) [63,64]. Thus, galectin-3 can affect the Wnt signalling pathway by co-operating with β-catenin to activate the transcription of genes in a TCF/LEF-dependent manner [63,64]. Shi et al [65] recently emphasized the role of galectin-3 in this pathway: inhibition of galectin-3 or Wnt-2 by siRNA silencing inhibited TCF/LEF-reporter activity and decreased the level of cytosolic β-catenin in colorectal cancer cells containing downstream mutations. Moreover, inhibition of both Wnt-2 and galectin-3 has synergistic effects on suppressing the canonical Wnt signalling pathway and causing apoptosis [65].

**Growth-regulatory signalling pathways**

Galectin-1 exerts anti-proliferative activity in epithelial tumour cell lines. This requires interaction with the α5β1 integrin and results in inhibition of the Ras–MEK–ERK pathway and in increased SP1- and SP3-dependent transactivation of p27kip1 as well as p21Waf1 (Figure 2) [66]. In α5-deficient HT29 colon carcinoma cells, galectin-1 has no effect on proliferation or p27kip1 and p21Waf1 expression, while the effect is restored by stable transfection of α5 [66]. On the other hand, active H-Ras and K-Ras are binding partners of galectin-1. These interactions with H-Ras or K-Ras were shown to enhance and extend the EGF (epidermal growth factor)-induced increases in Ras-GTP as well as Raf-1 and ERK (extracellular signal-regulated kinase) activity (Figure 2) [53]. Finally, it should be noted that like galectin-1, galectin-3 appears to interact with K-Ras, resulting in its stabilization in an active state (Figure 2) [67].

As seen above, galectin-3 can affect cell proliferation by regulating the Wnt pathway. In addition, in breast carcinoma cells, ectopic expression of galectin-3 leads to the activation of the cyclin D1 promoter, through several regulatory elements, including SP1 and CRE sites, revealing a growth-promoting activity for nuclear galectin-3 (Figure 2) [68]. Moreover, in the case of anoikis, galectin-3 transfectants upregulate the cell cycle inhibitors p21Waf1 and p27kip1, instead of undergoing anoikis (Figure 2). It was proposed that galectin-3 is able to drive the cells to a growth arrest at an anoikis-insensitive point, resulting in anchorage-independent cell survival. This feature of galectin-3 could be important in relation to its metastasis-promoting activity [69].

**Apoptosis regulation**

Galectin-3 enhances cell resistance to a variety of apoptotic stimuli, including UVB, TNF-α/cycloheximide, genistein, nitric oxide, cisplatin, etoposide, doxorubicin, and loss of anchorage [70,71]. First of all,
Figure 1. Involvement of galectin-3 in the Wnt signalling pathway. Galectin-3 is a known binding partner of β-catenin and, like β-catenin, can be phosphorylated by both CK1α and GSK-3β, the latter being promoted by interaction of galectin-3 with axin. Phosphorylation of galectin-3 by CK1α on Ser6 is also important in controlling its intracellular localization. In the nucleus, galectin-3 binds to the complex formed by β-catenin and TCF/LEF and co-operates with β-catenin to activate the transcription of Wnt target genes in a TCF/LEF-dependent manner.

Figure 2. Involvement of galectin-1 and -3 in growth-regulatory signalling pathways. Binding of galectin-1 to active H-Ras stabilizes its membrane anchorage and results in the clustering of active H-Ras and galectin-1 in non-raft microdomains. Galectin-1 also binds active K-Ras, although affinity for H-Ras is higher. Galectin-1 augments and prolongs Ras activation and shifts the interaction of the activated molecule away from PI3K and towards Raf-1. Galectin-3 also interacts with K-Ras. Interaction between galectin-1 and the α5β1-integrin results in inhibition of the Ras–MEK–ERK pathway and induction of p21\(^{\text{Waf1}}\) and p27\(^{\text{kip1}}\). Nuclear galectin-3 stabilizes the transcription factor SP1 and induces cyclin D1 promoter activity. Lastly, galectin-3-overexpressing cells respond to the loss of adhesion by inducing a G1 cell-cycle arrest through the up-regulation of p21\(^{\text{Waf1}}\) and p27\(^{\text{kip1}}\).
Figure 3. Role of galectin-3 and -7 in apoptosis regulation. Galectin-3, a p53-repressed gene, enhances cell resistance to various apoptotic stimuli. In contrast, galectin-7 is among the many genes induced by p53, such as the death receptor KILLER/DR5 that participates in the apoptosis programme orchestrated by the tumour suppressor. Galectin-3 also regulates mitochondria-related events during apoptosis and was shown to relocalize to perinuclear mitochondrial membranes upon apoptosis induction, as well as to bind to Bcl-2. Galectin-3 can also affect apoptosis by acting on the PI3K/Akt pathway and, more specifically, by modifying the level of activation of Akt.

galectin-3 is a p53-repressed gene. In an elegant study, Cecchinelli et al [72] showed, in RKO and HCT116 colon carcinoma cells, that p53 becomes activated in response to UV treatment following phosphorylation by the kinase HIPK2 on Ser46, this phosphorylation being important for repression of galectin-3 expression at the transcriptional level (Figure 3). Moreover, repression of galectin-3 by p53 markedly enhances p53-dependent apoptosis [72].

With regard to the anti-apoptotic function of galectin-3, its phosphorylation at Ser6 is crucial since it regulates its nuclear/cytoplasmic shuttling [33,73,74]. Indeed, cytoplasmic, but not nuclear, galectin-3 displays anti-apoptotic activities: in response to an apoptotic insult, such as cisplatin treatment, wt galectin-3 is exported out of the nucleus and relocates to perinuclear mitochondrial membranes. In the opposite, a point mutant where Ser6 has been replaced by Ala fails to be exported to the cytoplasm and to protect from apoptosis [73,74]. The anti-apoptotic role of galectin-3 at the mitochondria is at present poorly understood. In addition to Ser6 phosphorylation, interaction of galectin-3 with synexin, a Ca^{2+} and phospholipid interacting protein, is important for its translocation to the perinuclear mitochondrial membranes and subsequent inhibition of cytochrome c release (Figure 3) [73]. Moreover, galectin-3 contains the NWGR motif found in the BH1 domain of different Bcl-2 family members and was shown to interact with Bcl-2, although the significance of this interaction is unknown (Figure 3) [69,75,76].

Lastly, galectin-3 can affect apoptosis by acting on the PI3K/Akt pathway. Ectopic expression of galectin-3 in J82 bladder carcinoma cells confers resistance to TRAIL which is correlated with an increased level of active/phosphorylated Akt: active Akt is able to block BID cleavage, an important event with regard to the permeabilization of the mitochondrial outer membrane during apoptosis (Figure 3) [77]. However, the effect of galectin-3 on TRAIL-induced cytotoxicity is complex and cell-type-specific, since other studies showed that ectopic expression of galectin-3 in BTS49 breast carcinoma cells sensitizes to TRAIL-induced apoptosis which is correlated to a lower level of Akt activation [78,79].

Galectin-7 (PIG1, p53-induced gene 1) is highly inducible by p53 and is pro-apoptotic: in DLD-1 colon carcinoma cells and Hela cells, ectopic expression of galectin-7 enhances apoptosis in response to stimuli such as hypoxia, etoposide, TNF-α/cycloheximide or camptothecin [80,81]. Transfectants showed enhanced cytochrome c release, caspase-3 activation, and PARP

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cleeage (Figure 3) [80]. Galectin-7 appears to exert its pro-apoptotic function intracellularly, upstream of cytochrome c release and JNK activation [80].

**Potential involvement of galectins in clinical and therapeutic tools**

As detailed above, the data collected on altered galectin expression in the diseased digestive tract offer various potential diagnostic and prognostic applications in surgical pathology. The more abundant and promising data concern galectin-3 in colorectal cancers. As shown in Table 2, immunohistochemistry is the most used technique to evaluate galectin expression. This method has the advantages of preserving the morphological aspect of the analysis and of using cost-effective techniques which are routinely in use in pathology laboratories. However, this approach still demands substantial improvements in terms of standardization of both the immunohistochemical procedure (eg with regard to the different available antibodies) and the staining evaluation (eg by defining adapted scoring systems). More particularly, the evaluation system has to integrate the identification of the specific staining locations in terms of cell type (epithelial, stromal, inflammatory cells) and cellular location (nuclear, cytoplasmic or extracellular) because these different locations involve different biological roles played by galectins in the various digestive tract diseases. Furthermore, a panel of galectins seem involved at different disease stages. Consequently, the development of useful tools in practical surgical pathology requires the integration of expression evaluation for different galectins (eg galectin-1, 3, 4, and 8), possibly together with a number of their respective ligands or partners, such as those involved in the signalling pathways described above. In complement to the immunohistochemical approach, assessing galectin levels in serum also offers interesting perspectives as diagnostic tools. Finally, it should be kept in mind that all the retrospective data supporting potential clinical applications require validation in prospective studies.

In addition to the diagnostic and prognostic impact, an improved evaluation of the expression of galectins and their partners will also have positive consequences on the development of efficient therapeutic tools, particularly in the field of targeted therapy. The literature reports preliminary but encouraging results related to therapeutic approaches targeting the galectin systems. Two of them have already been mentioned in direct connection with the signalling pathways described above. The first concerns siRNA silencing of Wnt-2 and/or galectin-3 expression which results in apoptosis induction in human colorectal cancer cells [65]. The second extrapolates a decrease in chemotherapy resistance after down-regulation of intracytoplasmic galectin-3 by a siRNA approach, as investigated for other cancer types with respect to cisplatin and etoposide, which are largely used in colon cancer therapy [71]. Another therapeutic opportunity concerns the roles played by galectin-1 and -3 in the tumour endothelium and their consequences in terms of metastasis and angiogenesis [82]. Indeed, *in vitro* studies reveal that the use of anti-galectin-1 antibodies might induce angiostatic effects as well as prevent heterotopic adhesion between endothelial and tumour cells and thereby prevent metastasis [82]. Other authors focus on the importance of dietary carbohydrate compounds as agents for prevention and/or treatment of the cancer. Indeed, Nangia-Makker *et al* [83] have demonstrated *in vitro* and *in vivo*, using breast and colon cancer models, that modified citrus pectin inhibits carbohydrate-mediated angiogenesis by blocking the association of galectin-3 to its receptors. Finally, the role of galectin-1 in the immune response also offers interesting perspectives. The group of Rabinovich supports the concept that both in the tumour and in the tumour-associated stroma, galectin-1 plays a potent immunosuppressive role that may be a useful therapeutic target [56]. In contrast, this immunosuppressive ability could be potentially useful in treating diseases involving deregulated T-cell activation, such as Crohn’s disease. In this context, galectin-1, which is an endogenous lectin that is neither immunogenic nor cytotoxic, would have distinct advantages over the currently used unsselective immunosuppressive agents [42]. Finally, another perspective concerns the target of galectins expressed at cancer cell surfaces for targeted delivery of conventional anticancer drugs to improve their efficacy and tolerance. A phase II trial with colorectal cancer patients is presently underway with 5-fluorouracil in combination with DAVANAT® (Pro-Pharmaceutical, Inc.), a carbohydrate polymer that is composed of mannose and galactose and hypothesized to target cell surface galectins (see http://clinicaltrials.gov/; identifier: NCT00388700).

**Conclusions**

Glycobiology has become an important extension of modern molecular biology, given the multifunctional capacity of galectins. With respect to the digestive tract, most studies so far have focused on the diagnostic and prognostic values of galectin-3 and, to a lesser extent, galectin-1 and -8 in colorectal cancer. Research on a potential role for galectins in inflammatory bowel diseases is also developing and certainly deserves further attention since these molecules are also involved in immune homeostasis and inflammation.

In view of the available data, the galectin system offers great potentialities in terms of diagnostic and prognostic tools. However, their concretization requires further advances in the standardization of both immunohistochemistry and evaluation systems. More particularly, the evaluation systems have to take into account the specificities of the galectin expression patterns which are in direct relation with their biological functions. A reliable characterization
of galectin expression patterns in individual cancers may also play an essential role in the development of anti-cancer treatments and, more particularly, targeted therapies. First results on the challenging design of specific galectin inhibitors suggest that such molecules with subtle differences in carbohydrate structures may be potentially used to specifically block different steps of tumour growth, angiogenesis, and metastasis. This promises future scenarios in which members of the galectin family and/or their ligands will be used as therapeutic modalities for neoplastic as well as inflammatory disorders. However, further advances are required to efficiently concretize the therapeutic potential of these multifunctional molecules because of the complexity and the variety of the roles played by galectins in digestive diseases and for which controversies remain.

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Teaching materials

Power Point slides of the figures from this Review may be found at the web address http://www.interscience.wiley.com/jpages.0022-3417/supppmat/path.2334.html

References

Galectins in digestive diseases


