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Context-dependent multifunctionality of galectin-1: a challenge for defining the lectin as therapeutic target

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Introduction: One route of translating the information encoded in the glycan chains of cellular glycoconjugates into physiological effects is via receptor (lectin) binding. A family of endogenous lectins, sharing folding, a distinct sequence signature and affinity for β-galactosides (thus termed galectins), does so effectively in a context-dependent manner.

Areas covered: An overview is given on the multifunctional nature of galectins, with emphasis on galectin-1. The broad range of functions includes vital processes such as adhesion via glycan bridging, glycoconjugate transport or triggering signaling relevant, for example, for growth regulation. Besides distinct glycoconjugates, this lectin can also interact with certain proteins so that it can target counterreceptors at all sites of location, that is, in the cytoplasm and/or nucleus, at both sides of the membrane or extracellularly. Approaches to strategically exploit galectin activities with therapeutic intentions are outlined.

Expert opinion: The wide versatility of sugar coding and the multifunctionality of galectin-1 explain why considering to turn the protein into a therapeutic target is an ambitious aim. Natural pathways shaped by physiologic master regulators (e.g., the tumor suppressor $p16^{INK4a}$) are suggested to teach inspiring lessons as to how the lectin might be recruited to clinical service.

Keywords: apoptosis, glycosylation, lectin, sialylation, stroma, tumor suppressor

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1. Introduction

Cell sociology does not operate by proteins and their interactions alone. The ubiquity of glycosylation draws attention to the sugars attached to proteins and lipids. After first having successfully mastered the enormous task to characterize the complex structures of the glycan part of cellular glycoconjugates, the large set of saccharides detected indicated that glycans can do much more than altering solubility or other physicochemical parameters of their scaffolds. Like peptide sequences they can be bioactive code words based on the sugar alphabet [1]. Ensuing work revealed that the implied potential to store and transmit biological information is indeed realized, and this in different ways [1]. One route is the translation of the sugar-encoded information into effects by sugar receptors, termed lectins [2]. Furthering acceptance of the concept of the sugar code are the growing knowledge about lectin evolution and the ubiquitous occurrence of sugar receptors. Not just few proteins have developed the capacity to specifically bind sugars. In fact, at least 14 different folds are known to form carbohydrate-binding sites ranging in design from shallow groves to rather deep pockets, in each family with structural variations

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Article highlights.

- Glycans of cellular glycoconjugates are biochemical code words, which are translated into effects by binding receptors (lectins).
- Galectins, one of the least 14 families of animal and human lectins, are defined by sharing a common fold (β-sandwich), a sequence signature and binding to β-galactosides.
- Members of this lectin family have a broad functional versatility, via interplay with distinct counterreceptors, for example, in adhesion, growth regulation and transport processes.
- Galectin-1, a homodimer with potent cross-linking activity, is present at different sites in the cell and can also be secreted in a non-classical manner to act extracellularly.
- Distinct cellular glycoconjugates (e.g., the glycoproteins fibronectin and its integrin receptor or ganglioside GM1) in suited topological presentation and also proteins act as counterreceptors.
- Regulatory control of tumor growth (e.g., exerted by a tumor suppressor) highlights the potential of glycan reprogramming on galectin functionality.
- Areas of concern are identified to give research a clear direction for rigorously testing the perspective of galectin-1 as therapeutic target.

This box summarizes key points contained in the article.

of the common motif by genetic divergence [2]. What at first may have seemed bewildering owing to the sheer complexity of the lectinome has now been given a clear order by classification according to sequence signatures. Their definition reflecting homology facilitated to systematically scour data banks for further group members fitting the given criterion.

Functional assays such as the classical hemagglutination test used by Mitchell [3] or Stillmark [4] in their pioneering studies in the 19th century and affinity chromatography introduced by Agrawal and Goldstein [5] are invaluable tools to track down lectin activity and purify respective proteins. Whereas plant lectins (hemagglutinins), especially because of the ability of some prominent members of this superfamily to distinguish blood groups and the rich yields from easily available sources such as seeds, have a fairly long history as tools for glycophenotyping in medical research [6-8], work with endogenous (mammalian, human) lectins was in the beginning hampered by comparatively small yields, for example, from tumors or tumor lines [9-11]. This situation was fundamentally changed by cloning and recombinant production. These technical breakthroughs paved the way for making definitive structural studies possible. Hereby, a rational classification system based on molecular relationship in terms of sequence and folding was established, a formal step aiding to address the question whether lectins can become therapeutic targets.

Encouragingly, the term 'sugar code' implies a broad range of sugar-based activities governed by the interplay of glycans with receptors, as is the case [1], and numerous examples underline the connection of aberrations in glycan synthesis to diseases (in animal models and clinically) and the potential for drug design in this area [12-14]. With respect to cellular interactions, lectins targeting readily accessible branch-end epitopes of glycan chains, mostly β-galactoside derivatives, are of particular interest. To no surprise, several families of endogenous lectins, with marked intrafamily diversity [15,16], are known to bind such determinants, among them the galectins.

2. Galectins: regulators of adhesion, growth and more

The key to the first isolation of a galectin, from the electric organ of *Electrophorus electricus* (electric eel, thus called electrolectin), was the presence of reducing agent in the buffer to maintain lectin activity by protection against oxidation [17,18]. Hemagglutination studies on extracts of different rat and chicken organs, also of murine neuroblastoma (N-18) cells [17], later extended to seven human and murine tumor cell lines (one adenocarcinoma and one fibrosarcoma line as well as five melanoma lines, from which one line was negative in assays, an early indication for biological heterogeneity between lines obtained from the same tumor entity) [19], pointed to presence of such β-galactoside-specific proteins in mammals and also tumors. Using first epichlorohydrin-treated agarose, later conjugation of lactose or a β-galactoside-presenting glycoprotein (especially asialofetuin) as ligand to the resin in affinity chromatography, as done for plant lectins, verified this assumption [9,11,17,20]. What now qualifies a lectin to become a member of the galectin family is to share affinity for β-galactosides and the β-sandwich folding pattern with a characteristic sequence signature around the central tryptophan (Trp) residue (here the amino acids responsible for the network of hydrogen bonding are listed) [18]. Of note, the inherent sequence homology among these proteins will inevitably impose restrictions to the design of galectin-specific inhibitors. The common folding, position of the carbohydrate-binding site and its architecture are shown in **Figure 1A** for the case of the homodimeric galectin-1. A network of hydrogen bonds, especially involving the hydroxyl groups at the axial 4′-position (explaining the specificity to galactose vs mannose/*N*-acetylglucosamine) and the exocyclic 6′-position (hereby excluding 6′-substituted moieties such as 6′-sialylated branch ends from becoming a ligand) of galactose, and the C-H/π-interaction between the B-face of the amphiphilic sugar and the indole ring of the Trp moiety govern the target selectivity of recognition [2]. The involved amino acids (highlighted in the left subunit of the homodimer in **Figure 1A**) form the sequence signature, which is instrumental to trace new family members, as noted already above.

In addition to sequence variations around the recognition site (please note that changes far apart can also affect the thermodynamics of binding as shown for engineered variants of

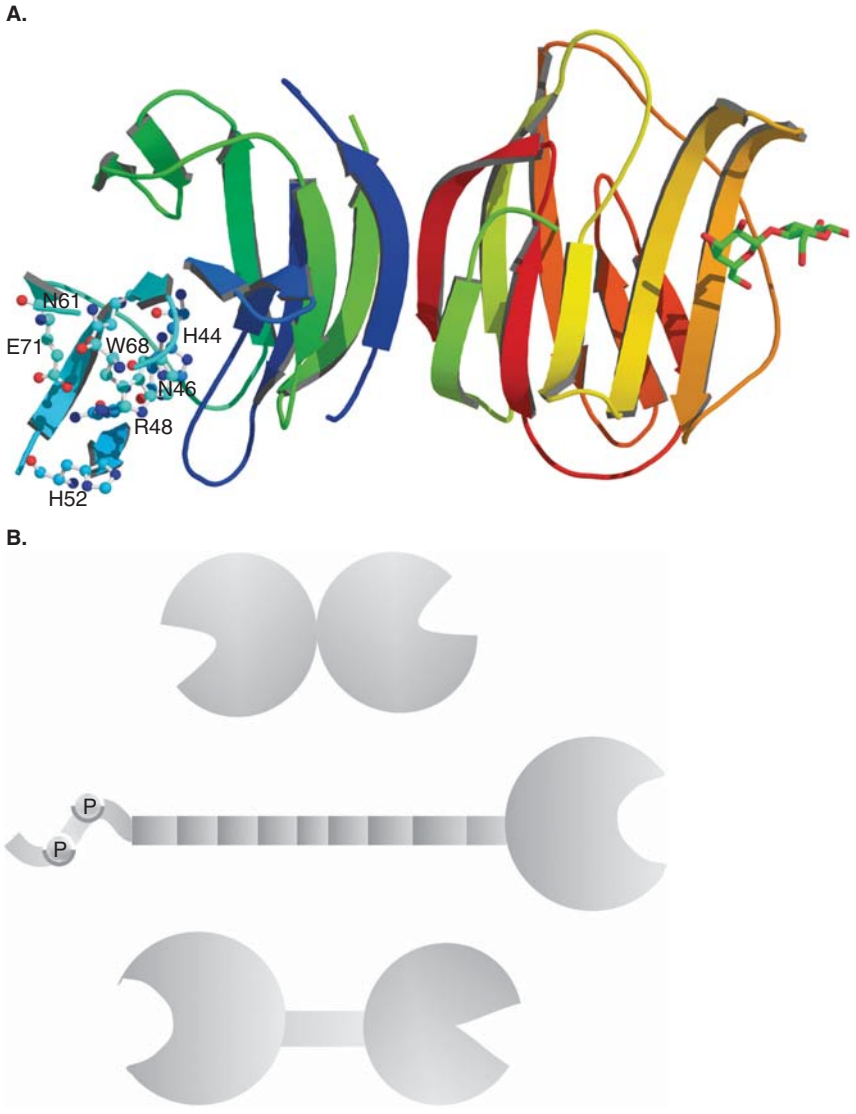

Figure 1. A. Illustration of the β-sandwich fold of homodimeric galectin-1 [21], with localization of the contact site for lactose (right) and the amino acids crucial for binding (left). **B.** Schematic illustration of the three types of lectin-site presentation in the galectin family. The prototype design consists of two identical non-covalently associated protein subunits, the chimera-type galectin-3 of the C-terminal carbohydrate recognition domain connected to nine collagen-like repeats and an N-terminal sequence with two sites for serine phosphorylation and the tandem-repeat-type design of two different subunits held together by a linker peptide, for example, 42 amino acids for galectin-4 and 28 or 70 amino acids for the two forms of galectin-8 arising from alternative splicing [23]. Of note, the three types of structural design endow the proteins with different cross-linking capacities.

human galectin-1 [21]), galectins differ in the display of their lectin sites [22-24]. As illustrated in **Figure 1B**, the non-covalent design of the prototype proteins is complemented with chimera-type (a lectin domain attached to a collagen-like section with proline/glycine-rich repeats and a terminal sequence harboring sites for serine phosphorylation: galectin-3) and tandem-repeat-type modes of structural organization. The covalent connection by a linker peptide facilitates to bring together two lectin sites in a topology likely depending on the properties of the individual linker. Interestingly, alternative splicing engenders production of variants with different linker lengths, and this with distinct organ expression profiles in mammals and birds [23,25].

Looking closely at galectin expression in animals, occurrence of these three subgroups is phylogenetically conserved [23,24]. Considering the abundance of β-galactosides on cellular glycoconjugates, many candidates are in principle available as docking sites for lectins. The key question is

how the galectins achieve their target specificity to become meaningful physiological effectors. Apparently, the combination of reacting with distinct counterreceptors together with the ability to form aggregates (lattices) is the molecular basis for properly triggering the diverse functional consequences of galectin–glycan interaction [24,26,27], examples presented in **Figure 2**. Particularly well studied is the involvement of galectins in inflammation. The elucidation of mechanisms to drive activated T cells into apoptosis, first for galectin-1 followed by respective analyses of galectins-2, -3, -4, -7, -8 and -9, has revealed different ways toward the same final destination for the members of family [28,29]. This clear evidence for non-redundant functionality among galectins intimates distinct patterns of production. Indeed, galectins show cell-type specificity; cells and tissues can express several galectins at the same time, as documented by immunohistochemical/RT-PCR (reverse transcriptase-polymerase chain reaction) fingerprinting, so that these lectins' activities should be considered to be embedded into a network, with emerging possibilities for functional antagonism or cooperation [2,24,29-33]. As the terminology let us assume, galectin-1 is at present the most thoroughly studied family member (for overview on the biophysical characterization of the protein and its interaction with ligands, please see Figure 4 in [2]). This work viewed in aggregate leads to the conclusion that galectin-1 is a pleiotropic effector, operative at different sites within the cell, at the membrane and extracellularly after secretion.

3. Galectin-1: a multifunctional protein

Galectin-1, encoded by a single gene, is translated on free cytoplasmic ribosomes and can then take a non-classical route of secretion [23,34,35]. Alternatively, galectin-1 can remain cytoplasmic or even move to the nucleus, for example, to become a part of the nuclear machinery for pre-mRNA splicing [36,37]. An illustration for cytochemical localization of human galectin-1 in the cytoplasm is given in **Figure 3A** (along with visualization of reactive sites in the nucleus with labeled galectin-1 and a control reaction for immunodetection of galectin-3 in **Figure 3B and C** to show a case of differential expression). The profile of galectin-1 presence is known to deviate from house-keeping proteins by cell-type specificity and potential for marked regulation, its extent depending on the cell type and developmental state [18]. An instructive example for a malignancy-associated increase of production, together with a shift in intracellular localization on progression, has been obtained by analysis of hypopharyngeal/laryngeal squamous cell carcinomas [38]. Functional correlations for cell growth/immune regulation have been intimated by the 10-fold increase in T cells of the CEM C7 leukemia line on glucocorticoid exposure [39] and an about threefold upregulation in $CD4^+CD25^+$ T regulatory cells by activation, the lectin thereafter seen in nuclei and at the inner side of the plasma membrane [40,41]. Besides such (not yet defined) physiological mechanisms on gene regulation and the strong association with differentiation in several organs [18], galectin-1 expression can be enhanced by differentiation-inducing agents such as butyrate (2 mM), apparently under the control of a 5′-proximal Sp1 site in the promoter of the galectin-1 gene [42,43]. Such reagents, along with compounds reducing DNA methylation or inhibiting histone deacetylase [44-46], offer the possibility to manipulate the galectin level.

A further level to control mammalian galectin-1 is via reversible redox reactions involving its cysteines (Cys; six in the human protein), which abolish its lectin activity under oxidative conditions [47-49]. The resulting oxidized form has acquired the ability to block cell growth or promote axonal regeneration via carbohydrate-independent mechanism(s) [50-52]. Remarkably, the avian ortholog CG-1B, the second galectin showing this particular sensitivity via cysteines, maintains its lectin activity despite the redox reactions, which lead to the formation of intra- or intersubunit disulfide bridges involving Cys2/Cys7 [49,53].

With respect to its lectin activity, a broad reactivity is intuitively expected with cellular glycoconjugates, because β-galactosides are a common feature of glycoproteins, glycolipids and glycosaminoglycan chains such as keratan sulfate. As already noted above, the presence of a suitable glycan determinant appears to team up with topological factors dealt with systematically previously [2], to help limit the number of binding partners. As a consequence, only distinct glycoconjugates (and proteins) are reactive, **Table 1** listing defined compounds from various cell types/contexts. Obviously, the lectin is capable to choose certain molecules from the classes of glycolipids, glycoproteins and glycosaminoglycans for the molecular rendezvous. For instance, the presentation in microdomains is a salient factor to turn the pentasaccharide of ganglioside GM1, which contacts the lectin by carbohydrates in both of its branches, into a high-affinity docking site on the surface of neuroblastoma (SK-N-MC) cells [54,55]. This apparent cooperation of structural and topological factors (ligand structure, presentation) will then be responsible for achieving precision in initiating post-binding signaling, in contrast to a 'simple' interaction of a peptide/protein to its receptor.

In view of the galectins' cytoplasmic/nuclear presence, it is fitting that binding partners are known in these compartments, too (**Table 1**). Mechanistically, a respective complex formation is relevant for spliceosome assembly or for properly guided positioning to promote downstream signaling (for oncogenic H-Ras), in the latter case a site independent from the contact area for glycans being used for the association as shown by testing the L11A mutant [56,57]. This feature, that is, to involve different regions of the protein for different functions, is not uncommon among lectins (e.g., two sites used for transport/cell–matrix interaction of discoidin I [58]) and lets them join the list of moonlighting proteins [59,60]. As stated, 'the existence of moonlighting functions complicates efforts to understand metabolic and regulatory networks, as well as physiological and pathological processes in organisms' [60].

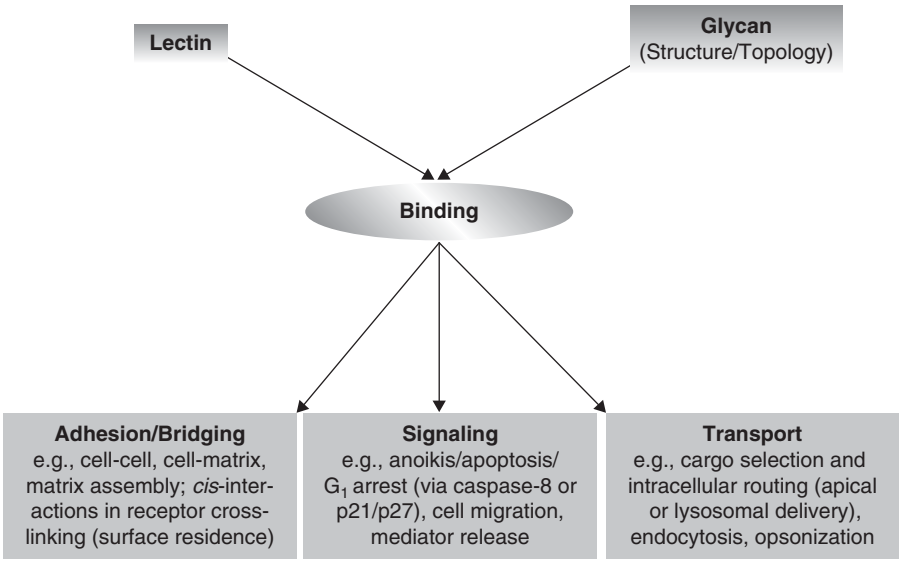

Figure 2. Examples for the translation of glycan-based information into effects by lectins, focusing on events elicited by human galectins.

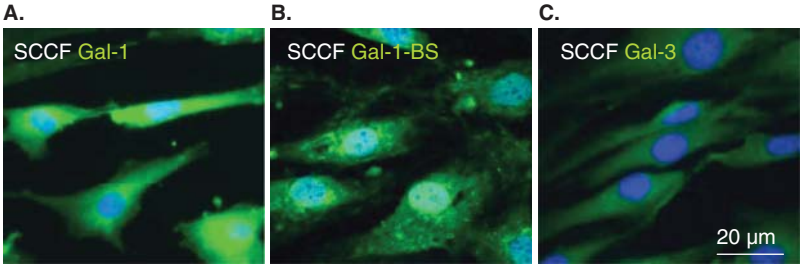

Figure 3. Cytochemical detection of galectin-1 (**A**), binding sites for galectin-1 (**B**) and of galectin-3 (**C**) in fibroblasts isolated from a human squamous cell carcinoma.

The fact that specific characteristics of the glycophenotype determine the cellular responsiveness to galectin-1 underlies the information on growth regulation given in the central part of **Figure 2** within 'signaling'. In detail, anoikis induction in pancreas (Capan-1) carcinoma cells under the control of the tumor suppressor $p16^{INK4a}$ rests on reprogramming protein expression (e.g., upregulation of α_5-integrin subunit) and glycosylation (e.g., reducing glycoprotein α2,6-sialylation by throttling sialic acid biosynthesis) and shifting the balance of galectin expression to galectin-1 (please see **Figure 4** for an overview on involved reactions) [61-63]. In more general terms, galectin-1 appears to be a part of the control system for epithelial integrity, driving cells either into anoikis (involving caspase-8) or G_1 arrest (involving upregulation of cyclin-dependent kinase inhibitors p21 and p27), unless the glycans of the functional counterreceptor (here $\alpha_5\beta_1$-integrin, the fibronectin receptor) are masked by α2,6-sialylation [64,65]. The same holds true for the induction of apoptosis in activated T cells, a reaction in which CD7, CD43 and/or CD45 can play a role [28,29,66,67]. Intriguingly, a certain carbohydrate determinant, that is, the core 2 mucin-type *O*-glycan modification (for structural details on *O*-glycans, [68]), partakes in T-cell apoptosis, as it does when galectin-1 reduces growth of prostate (LNCaP) cancer cells [69,70]. Enzyme regulation to favor core 2 branching thus renders these cells reactive to galectin-1.

For neuroblastoma (SK-N-MC) cells, the functional counterreceptor (i.e., ganglioside GM1) is made available by a different pathway of glycome reprogramming: enhancing cell surface ganglioside sialidase activity to convert the ganglioside GD1a into GM1 by removing one sialic acid moiety [71,72]. Similarly, activation of T effector cells causes increased cell surface presentation of this ganglioside, along with a distinct type of Ca^{2+} channel (TRPC5) [73]. These events explain an aspect of how T regulatory cell-derived galectin-1 sets limits to T effector cell activity. The lectin's surface presentation/secretion is upregulated by

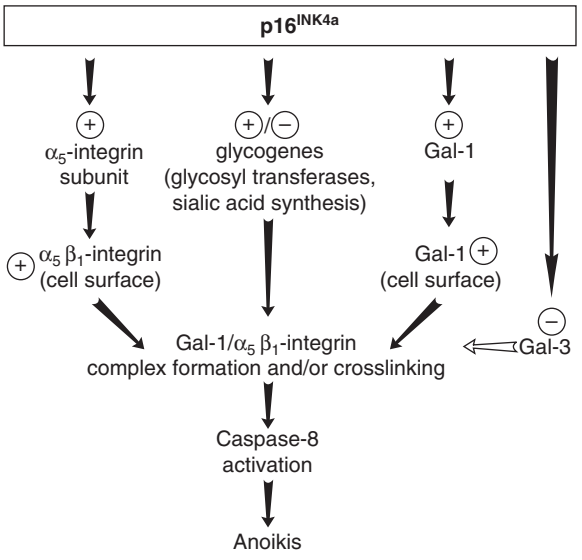

Figure 4. Schematic illustration of the mechanisms involved in anoikis induction by the tumor suppressor p16^{INK4a} in human pancreas (Capan-1) carcinoma cells. The orchestration of galectin-1 and integrin upregulation with reprogramming glycosylation underlies caspase-8 activation [61-63]. Functional antagonism by galectin-3, which can block galectin-1 binding to the cell surface, is minimized by its down-regulation [62]. At the same time, this decrease has consequences for the following aspect intracellularly: galectin-3 in the cell binds oncogenic K-Ras, also a target of p16^{INK4a}-dependent downregulation, and hereby activates its downstream signaling [126,127].

activation [41], as it is in the case of the neuroblastoma cells [71], the ensuing galectin-1/GM1 interplay causes the Ca^{2+} level in T effector cells to move up, hereby committing the cells to anergy/apoptosis [73,74]. Deficiency in this system, for example, insufficient GM1 presentation seen in T cells of a murine diabetes model, can be relevant for autoimmune diseases and has been corrected *in vitro* by applying the ganglioside [74].

Since T regulatory cells can also dampen immune responses in tumors, here likely with negative consequences, it is noteworthy that intratumoral presence of galectin-1 and these T cells has been described as a negative prognostic factor in hepatocellular carcinoma [75]. Within tumors, *in situ* presence of galectin-1 can affect other inflammatory cells besides T effector cells (e.g., tumor-associated dendritic cells instructing them in the case of lung (A549/H460) cancer cells to produce and release heparin-binding EGF-like growth factor [76]) and also fibroblasts. Acting on fibroblasts, the lectin can mediate their conversion to myofibroblasts in the tumor stroma. These cells then have the capacity to steer the microenvironment toward favoring tumor progression [77-79]. Presence of galectin-1 and cancer-associated stromal cells positive for α-smooth muscle actin is illustrated in **Figure 5A**, as is the occurrence of myofibroblasts in a culture of normal human dermal fibroblasts after being exposed to galectin-1 (**Figure 5B**). If encountering a suited counterreceptor on tumor cells (tissue plasminogen activator in pancreas carcinoma [80]), auto- and paracrine routes for galectin-1 in the stroma-tumor transition are likely to promote tumor progression. As this example attests, galectin-1 is not only a negative growth regulator (via glycan binding or after oxidation), as described above. Its functionality spectrum also encompasses pro-proliferative (mitogenic) activity, smooth muscle cells and pulmonary arterial cells [81] or the human MPS fibroblast line [82] belonging to the set of responsive cell types. Since the mode of growth regulation can even depend on the concentration tested, it was concluded that 'unsurprisingly, in view of this background, the interpretation of the actions of galectin-1 in developmental situations, both normal and neoplastic, is often very complex' [83].

Obviously, galectin-1 has a wide array of activities, depending on the cell type, spatiotemporal context and availability of counterreceptor(s), in terms of suited glycosylation and protein expression. The major aim for a therapeutic manipulation is hitting the pathological aspect while avoiding harm at any physiologic site. Ideally, lectin therapy would thus spatially be strictly confined. The line of research toward this goal, initiated to be explored already two decades ago, is the application of the lectin by injection.

4. Galectin-1: the panel of therapeutic options

The lectin itself, first purified from the electric eel in 1983 and then the mammalian protein in the presence of dithiothreitol, has been tested *in vivo* in animal models of autoimmune disease. Starting with achieving amelioration of disease status in experimental autoimmune myasthenia gravis of rabbits [84], similar evidence was collected in various other animal models, for example, on autoimmune encephalomyelitis, type 1 diabetes and lupus erythematosus [85-87]. To figure out whether an analogous approach might work on tumors, in view of carbohydrate-dependent/independent growth-inhibitory effects of galectin-1 on certain cell types including tumor cells [50,51,64,65,82], which can be elicited/enhanced by compounds such as butyrate [88,89], the lectin's activity on cell types beyond the intended target needs to be taken into careful consideration: harming activated intratumoral T effector cells and generating (priming) progression-promoting stromal cells may shape a protumoral microenvironment. When focusing deliberation on tumor cells, the natural heterogeneity between subpopulations and by intertumoral variation may not be reflected adequately by testing one or few cell lines, or only lines with favorable features in culture, for example, rapidly growing cells. As a consequence, a panel of lines and histocultures will need to be monitored thoroughly prior to reaching reliable conclusions on benefit and safety.

Ways to block galectin-1 activity certainly encompass genetic knock-down. Tested on human glioblastoma (U87)

Table 1. Counterreceptors (glycoconjugate or protein) of galectin-1.

Glycan	CA125 (ovarian carcinoma antigen), CD2, CD3, CD4, CD7, CD43, CD45, CD95 (Fas), carcinoembryonic antigen (CEA), chondroitin sulfate proteoglycan, fibronectin (tissue), gastrointestinal mucin, hsp90-like glycoprotein, β_1-integrin (CD29), $\alpha_1/\alpha_4/\alpha_5/\alpha_7\beta_1$- and $\alpha_4\beta_7$-integrins, cell adhesion molecule L1, keratan sulfate, laminin, lamp-1, Mac-2-binding protein, nephrin, neuropilin-1, receptor protein-tyrosine phosphatase (RPTPβ), thrombospondin, Thy-1, tissue plasminogen activator, von Willebrand factor, distinct neutral glycolipids, ganglioside GM1
Protein	B lymphocyte adaptor molecule of 32 kDa (Bam32), $Ca_V1.2$ L-type calcium channel (α_1-subunit), Gemin4, oncogenic H-Ras, OCA-B, pre-B cell receptor (human, not murine system)

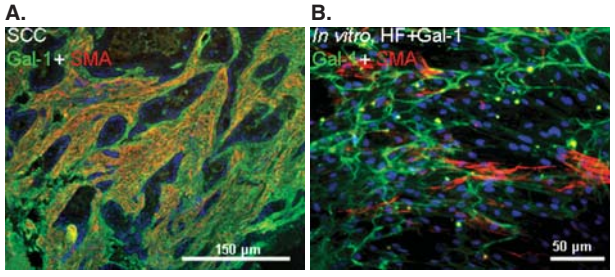

Figure 5. A. Strong signal intensity for galectin-1 in the extracellular matrix of the stroma of a human tonsillar squamous cell carcinoma and positivity for α-smooth muscle actin, indicative of conversion of stromal fibroblasts to cancer-associated fibroblasts. **B.** Exposure of normal human fibroblasts to galectins leads to production of galectin-1-containing fibers of extracellular matrix and conversion to α-smooth muscle actin-positive myofibroblasts.

cells, owing to the lectin's status as indicator of poor prognosis for this tumor entity [90], this procedure revealed the lectin to be connected to several aspects of the malignant phenotype. Its absence is associated with p53 increase and alterations in expression levels of a broad series of genes in adhesion/motility (91 from 631 spots on the chip surface) [91]. These results encourage further efforts to delineate comprehensively how induced deficiency alters the proteome. On the level of lectin interactions with cells, blocking either the carbohydrate recognition domain on the lectin or its ligand is a possibility. This can either be done with anti-galectin antibodies, as shown *in vitro* [10], or with galectin-1-derived peptides [92] and a monomeric (non-cross-linking) galectin-1 variant defective in triggering post-binding signaling. Exploiting differences in cross-linking capacities among galectins (**Figure 1B**) in cases with competition for the same cell surface counterreceptor, prototype galectin-1 activity will be impaired by presence of chimera-type galectin-3 (please see **Figure 4** for physiological regulation to optimize anti-tumoral galectin-1 functionality) [62,93]. Intracellularly, the design of a lectin mutant defective in binding oncogenic H-Ras seriously disturbed the proper delivery of the oncogene product, underscoring biomedical relevance for this type of carbohydrate-independent recognition [57]. Ligand derivatives offer a further option when interplay with glycans on the cell surface and/or in the extracellular matrix should be blocked. However, while synthetic tailoring generates compounds with activity in *in vitro* assays, as shown in **Figure 6**, and the combination of headgroup design with topology in glycoclusters is effective to increase selectivity [94], cross-reactivity of such a compound among the galectins and with members of other lectin families such as galactose-binding C-type lectins can compromise an assumedly helpful *in vivo* application. Consequently, systematic assays in settings of increasing complexity (biomedical relevance) are mandatory. Inevitably, systemic presence of an inhibitor will impair galectin activity not only at the desired site.

Interfering with binding can also operate on the level of the counterreceptor. The physiologic example of how the tumor suppressor $p16^{INK4a}$ makes the human pancreas (Capan-1) carcinoma cells susceptible to galectin-1-induced anoikis [61-63] is as inspiring as is the impact of cell type-specific regulation of relevant glycosyltransferases on galectin-1-induced apoptosis of activated T cells and a prostate cancer model (α2,6-sialyltransferase or core 2 *N*-acetylglucosaminyltransferase [67,69,70]). Directing glycosylation accordingly (here butyrate as inducer of galectin-1 expression mentioned above and as negative factor for α2,6-sialyltransferase activity shown *in vitro* [95] provides an instructive case study, as do glycophenotype changes after reconstitution of expression of tumor suppressors lost by microsatellite instability [96]) could thus follow natural precedents, although ways need to be found to reach cell-type specificity to avoid undesired reactions, for example, enhanced T cell death by galectin-1/core 2 *O*-glycan (on CD45) interplay [69]. Evidently, a cell and enzyme type-specific manner will be preferable to general and therefore wide-range interventions to glycan processing, in view of the concept of the sugar code. Equally of impact on galectin activity is the regulation of availability of the protein/lipid carrier for the decisive glycan determinant. The shift from $CD7^+/CD7^-$ cells to $CD4^+CD7^-$ leukemic T cells in patients during the progression of Sézary syndrome has been attributed to a galectin-1/CD7 (death pair)-dependent selection process [97]. Knowing the distinct counterreceptor (**Table 1**) can thus focus efforts to silence/booster expression of this binding partner or its post-binding signaling.

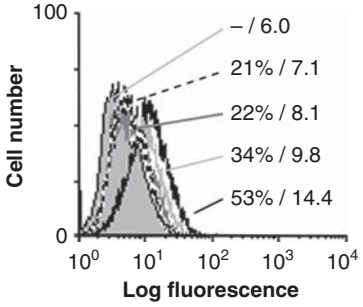

Figure 6. Semilogarithmic representation of fluorescent staining of tumor suppressor $p16^{INK4a}$-expressing pancreas (Capan-1) carcinoma cells by galectin-1 (10 μg/ml). Quantitative data are given for percentage of positive cells/mean fluorescence intensity, lines connecting these data with the respective staining profile. Given from bottom to top are the data for the control (100% value; no test compound present), the tests with 2 mM galactose, 2 mM lactose, 2 mM *p*-nitrophenyllactoside and the background control (0% value: no lectin added) [128]. A small positive effect by the addition of a β-anomeric aglycone is seen.

5. Conclusions

In principle, insights into the flow of bioinformation identify new therapeutic targets. Of course, the translation of sugar-encoded messages into effects by endogenous lectins attracts interest in this respect. Systematic searches for lectins and their characterization have delineated a wide panel of families with varying degrees of intrafamily diversity, elucidation of functions and interplay especially among proteins from the same family being a topic of current research.

A typical feature of galectins is their presence at different sites, within cells and/or outside cells, depending on the cell status (activation, differentiation) and cell type. The nature of the physiological response to a galectin critically depends on the presence of counterreceptors, with suited glycosylation and topology of presentation. The importance of spatial parameters can be inferred by testing panels of glycoproteins in specificity assays, and, indeed, the mode of presentation matters [98]. The examples on glycan reprogramming given above, in conjunction with upregulating galectin-1, attest orchestration of co-expression to be a crucial factor for optimizing the response, in intensity and specificity. This said, respective binding sites qualify as functional markers [99]. Detection of i) presence of these specific counterreceptors for tissue lectins and ii) operative binding will then let cell reactivity *in vitro* become predictable. Correlations of growth regulation with presence of the α_5-integrin subunit and reduced α2,6-sialylation in tumor suppressor-expressing pancreas (Capan-1) carcinoma cells [61-63] or with level of ganglioside GM1 presentation on activated T effector cells in a murine diabetes model [74] affirm this concept. Of course, this discovery of cell type-specific targets opens a wide research area, further underscored by evidence for prognostic relevance of galectin binding in head and neck (positive correlation) as well as lung (negative correlation) tumors (please see **Figure 3B** for an example of galectin cytochemistry) [100,101]. The identification of the nature of the counterreceptors and the rigorous structural characterization of galectin-reactive epitopes responsible for the target selectivity (in carbohydrate sequence, conformation and multivalency) will be the basis for developing rational approaches to interfere with pro-tumoral signaling initiated by the specific binding or to take advantage of growth-inhibitory capacity of galectin-1.

6. Expert opinion

Galectin-1 is a highly versatile protein. It is a potent modulator of diverse cellular activities at different sites of cells, depending on the given cellular features. Being a member of a family, it is embedded in a network, with possibilities for functional cooperation or antagonism. This situation raises intriguing questions to be answered. The lectin's pleiotropic character naturally accounts for a notably broad range of responses. They are triggered at different sites and in different cell populations, not all likely favorable for a patient. As consequence, manipulations of galectin parameters need to be rigorously put to the test in different models, among them certainly *in vitro* systems from cell lines to histocultures. When working with human cell lines, researchers should always reckon with the fact that a certain line naturally cannot serve as a model for an entire tumor entity. Moreover, considering the clinical level, it should not be overlooked that malignancies of a certain organ can develop via different routes. Taking colon cancer as a test case, chromosomal or microsatellite instability along with epigenetic silencing by CpG island methylation give rise to tumors, which are to be analyzed separately. As a step along this way, glycophenotyping *in situ* for microsatellite-stable/instable tumor cases has recently revealed intra- and intertumoral heterogeneity that may play into responses to galectins [102]. Thus, it is necessary to process a sufficiently high number of lines to gain an understanding of the actual reactivity profile *in vitro* (with proper consideration of the network), and these studies are highly informative to help assess the actual range of lectin activity. Preferably, they are linked to in-depth histopathological analysis of the tumor entity, which will also determine the relation of galectin parameters to presence of inflammatory cells and stromal expression.

At least two caveats should be kept in mind when working with animal models: i) the glycomic profiles between mammals can differ markedly, rendering extrapolations from one species to another problematic [103,104] and ii) the display and specificity of lectins can differ among species, for example, delineated in the case of the C-type asialoglycoprotein receptors of macrophages [105,106] and of liver [107], also for galectins [23,24]. Expression polymorphism for galectin-6, a tandem-repeat-type protein unique for mouse, has even been

detected between different strains [108]. On the level of the receptor, research on other glycan-interacting proteins has led to the conclusion that caution needs to be exercised when analyzing properties of overexpressed tagged forms of the protein cytochemically [109] or when deriving functional concepts from a single approach, here for a collectin based on induced deficiency versus *in situ* inhibition [110]. This also holds true for extrapolations from *in vitro* work to the *in vivo* situation: with respect to galectin-3, whose anti-apoptotic capacity has been assumed to generally favor tumor development/progression, the analysis of three mouse mutants, that is, two in the *Apc* gene (Apc^{Min} and Apc^{1638N}) and a *PyMT* transgenic line, which are models for genetically dependent origin of colorectal and mammary carcinomas, respectively, revealed no apparent consequence of this lectin's deficiency [111]. This observation was judged by the authors to be 'unexpected' [111]. Equally worthy of consideration, the broad-range perturbation after knocking-down galectin-1 expression referred to above [91] signifies that manipulating this protein's level can do (much) more to a cell than altering a single parameter, that is, silencing lectin expression.

Mutatis mutandis the same holds true for any envisioned manipulations of the glycomic profile. Inhibitors of glycan assembly or processing/maturation, which alter the glycan signature of cells markedly, may interfere with tumor cell growth *in vitro* but not reach the required *in vivo* efficiency and safety profile. On the grounds of the concept of the sugar code, rather drastic structural changes will inevitably trigger an array of consequences difficult to control. Here, hereditary diseases of glycosylation can teach salient lessons [12,112]. Already minor changes, for example, in β1,4-galactosyltransferase expression, may not only make their presence felt in lectin ligand production but also in levels of glycoproteins. Looking at expression of the EGF receptor as tumor-relevant parameter, an impact of the slight perturbation in β1,4-galactosylation characteristic for the Lec19 mutant of Chinese hamster ovary cells had been traced [113]. Beyond that, the multifunctionality of glycans (a case of moonlighting in the same, separate and overlapping sequence stretches) can turn a modification into a two(multi)-edged sword. Elevated core 2 *O*-glycosylation to switch on lectin-dependent growth regulation discussed for the LNCaP cells above [70], as possible downside, may mask tumor cells to let them pass by natural killer (NK) cells *en route* to metastasis formation [114]. Altering α2,6-sialylation, for example, to preclude binding of galectin-1 to glioblastoma cells as a means to set limits to lectin-dependent invasiveness [115], can implement other features characteristic of malignancy such as reduced adhesiveness. Moreover, it can translate into attenuating T and NK cell function via inhibitory siglec activity, for example, by siglec-9 [116]. Ideally, a rather subtle and functionally unambiguously effective reprogramming of counterreceptor(s) glycosylation should be accomplished, at best restricted locally. The case of the tumor suppressor $p16^{INK4a}$ sets a remarkable example in this respect.

In order to avoid disappointment in clinical testing, due to 'suboptimal preclinical validation' [117] or 'overly optimistic assumptions' [118], the target and the setting must be chosen critically. In this sense, it should be noted that 'reports of prognostic information on galectin-1 in tumors' were assessed to be 'few and inconsistent' [75]. Looking at this report on hepatocellular carcinoma in detail, specimens of 71 from 386 studied patients were positive for stromal cells without prognostic information and stratification into groups based on intensity of tumor staining disclosed prognostic relevance only at high level of galectin-1 expression [75]. Thus, the cohort of patients potentially benefiting from an anti-galectin-1 regimen will need to be precisely identified experimentally. Since nuclear presence of galectin-3, too, was an independent marker in this tumor type [119], indicating poor prognosis, while cytoplasmic galectin-9 signaled extension of survival time [120], a strong case is made for galectin-panel study. This suggestion is further backed by noting that the combination of simultaneous presence of galectins-1 and -4 provided the best survival prediction (very bad prognosis) for Dukes A/B colon cancer patients [32], whereas 5-year survival in bladder cancer correlated with galectins-2 and -8 [121]: galectin fingerprinting is therefore encouraged. The cases of functional antagonism between galectins-1 and -3 noted above epitomize the importance to appraise activity of a certain galectin in the concept of the network. In addition to assessing staining parameters in terms of intensity/percentage of positive cells, syntactic structure analysis, for example, distance between positive tumor cells or radius of cluster of positive tumor cells, proved its value together with galectin fingerprinting to delineate prognostic information, for patients with primary testicular cancer and lung metastases [122]. In the same line, to perform functional assays for more than one member of this family and for combinations of galectins *in vitro* to track down antagonism/synergy appears to be advisable.

Broadly speaking, a reasonable route therefore is to examine the galectin network more closely, in preclinical settings and on clinical material, to shape a guideline for singling out one or more members of this family in the given context for further study. As the combination of **Figure 2** with **Table 1** tells us, the galectin-dependent effects arise from the interaction with distinct counterreceptors. A galectin thus has diverse binding partners, for each target cell type apparently a specific set. Underscoring the respective part of the Section 5, the molecular definition of the determinants establishing the high-affinity sites on the corresponding glycoconjugates is the promising challenge to be mastered, and the techniques to do so are at hand [123,124]. The inherent differences in the nature of binding partners among cell (sub)populations and cell types make responses to galectin exposure more difficult to predict than cell reactivity for growth factors or cytokines. The dynamic glycan reprogramming, appealing as it is as efficient and sensitive switch for galectin reactivity, makes reliably measuring the responsiveness demanding. Context dependency of lectin action is not only known for the galectins; work on serum collectins, to give an example,

similarly pinpoints various variables to influence the final outcome in a complex manner [125]. With research discovering and characterizing the wealth of galectin activities and the cell type-selective counterreceptors as well as the correspondingly operative signaling pathways steadily making progress, proper disease states can likely be identified to test the idea of galectin-1 (alone or in combination) as therapeutic target.

Declaration of interest

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