Expert Opinion on Therapeutic Targets

ISSN: 1472-8222 (Print) 1744-7631 (Online) Journal homepage: https://www.tandfonline.com/loi/iett20

Galectin-3: is this member of a large family of multifunctional lectins (already) a therapeutic target?

Antonio Romero & Hans-Joachim Gabius

To cite this article: Antonio Romero & Hans-Joachim Gabius (2019): Galectin-3: is this member of a large family of multifunctional lectins (already) a therapeutic target?, Expert Opinion on Therapeutic Targets, DOI: 10.1080/14728222.2019.1675638

To link to this article: https://doi.org/10.1080/14728222.2019.1675638

Accepted author version posted online: 01 Oct 2019.

Publisher: Taylor & Francis & Informa UK Limited, trading as Taylor & Francis Group

Journal: *Expert Opinion on Therapeutic Targets*

DOI: 10.1080/14728222.2019.1675638

Galectin-3: is this member of a large family of multifunctional lectins (already) a therapeutic target?

Antonio Romero[1*] and Hans-Joachim Gabius[2*]

[1]Structural and Chemistry Department, Centro de Investigaciones Biológicas (CIB), CSIC, Ramiro de Maeztu 9, 28040 Madrid, Spain

[2]Institute of Physiological Chemistry, Faculty of Veterinary Medicine, Ludwig-Maximilians-University Munich, 80539 Munich, Germany

Corresponding author:

Dr. A. Romero (Research Professor of the Spanish Research Council, CSIC): Phone: +34 91 1098044, Fax: +34 91 5360432, e-mail: romero@cib.csic.es

Prof. Dr. Dr. h.c. H.-J. Gabius: Tel.+49-89-2180 2290, Fax: +49-89-2180 992290, e-mail: gabius@tiph.vetmed.uni-muenchen.de or gabius@lectins.de

ABSTRACT

Introduction: The discoveries that sugars are a highly versatile platform to generate biochemical messages and that glycan-specific receptors (lectins) are a link between these signals and their bioactivity explain the interest in endogenous lectins such as galectins. Their analysis is a highly dynamic field. It is often referred to as being promising for innovative drug design.

Area covered: We present a primer to the concept of the sugar code by glycan-(ga) lectin recognition, followed by a survey on galectin-3 (considering common and distinct features within this family of multifunctional proteins expressed at various cellular sites and cell types). Finally, we discuss strategies capable of blocking (ga)lectin activity, with an eye on current challenges and inherent obstacles.

Expert opinion: The emerging broad profile of homeostatic and pathophysiological bioactivities stimulates further efforts to explore galectin (-3) functionality, alone and then in mixtures. Like thoroughly assessing the pros and cons of blocking approaches for a multifunctional protein active at different sites, identifying a clinical situation, in which the galectin is essential in the disease process, will be critical.

Keywords

adhesion; apoptosis; galectin-3 (Gal-3); glycans; glycoproteins; polysaccharides

Article Highlights

- Glycan-lectin recognition is an efficient and versatile means of cellular information transfer.
- Members of the galectin family are emerging as multifunctional effectors in diverse cell types and at various sites of cells and tissues.
- Galectin-3 has a unique modular architecture suited for context-dependent aggregation and also interactions with glycans and with proteins.
- *In vitro* and *in vivo* work in models gives reason to assume correlations between galectin expression and disease states.
- Various approaches offer potential for blocking pairing of galectins with counterreceptor(s).
- Multi-site activity of multifunctional effectors, likely in a network, poses attractive challenges on the way to define the status of galectin-3 as therapeutic target.

1 Introduction

Molecular selectivity underlies first the detection and then the characterization of the activity profile of a large superfamily of (glyco)proteins that are called lectins. This term originates from work on blood group typing. Precisely knowing the blood group status is essential to predict and to preclude incompatibility of transfusions, thus fatal outcomes. The observations that components of plant seed extracts agglutinated erythrocytes depending on the type of blood group of the donor, exactly as natural serum antibodies do, not only prompted to coin the technical name ‚phytohaemagglutinin' but also „the word lectin from Latin *lectus*, the past principle of *legere* meaning to pick, choose or select" [1]. It highlights that proteins different from immunoglobulins can distinguish between cell surface epitopes, se*lectin*g their binding partners.

The following discoveries that simple sugars competitively inhibited lectin-dependent haemagglutination, that ABH(0) histo-blood group determinants and other types of blood group systems are oligosaccharides and that plant agglutinins can also precipitate polysaccharides and glycoproteins in a carbohydrate-specific manner gave reason to connect the term ‚lectin' with binding (receptor) capacity to carbohydrates (for review on the history of lectins, please see [2-4]). Since sugars have unsurpassed capacity to generate structural diversity in a minimum of size in oligomers [5], they are well-suited as a molecular alphabet to 'write' cellular signals, and this beyond the described phenotypic (signature-like) feature of erythrocytes. The known common abundance of glycoconjugates on cell surfaces with their high level of structural complexity (actually, glycans have indeed been called "complex carbohydrates" [6] or "complex heterosaccharides" [7]) and the fact that they "are much more complex, variegated and difficult to study than proteins or nucleic acids" [8] explain that the pace of progress in this field is necessarily slow in comparison. Presentation of elaborate structures at accessible positions, on surfaces and in the matrix [9,10], intimates that glycans are involved in information transfer. As the example of blood-group typing attests, they can be bioactive as ligands. Indeed, functional pairing of glycans with lectins as their receptors has become the tenet of the concept of the sugar code [11,12].

Fittingly, the toolbox for glycan synthesis and recognition is well equipped: a complex enzymatic machinery for glycan (ligand) assembly with many so-called glycogene products such as diverse families of glycosyltransferases and multiple folds for glycan recognition (by receptors), the currently accepted key characteristic for lectin classification, are at hand (for examples, please see [4,13-16]). In each category and case, the route via duplications and

ensuing sequence divergence in the coding and regulatory sections of the respective genes yielded groups of homologous proteins. They ensure fine-tuned signal ‚writing' in the sugar language and ‚translating' of these messages into widely diverse post-binding effects. The cyto- and histochemical application of plant and invertebrate lectins as laboratory tool to map the presence of cognate glycans substantiated the enormous potential of this class of receptors to pick certain epitopes of glycoconjugates from the cellular glycome [15,17].

The success of this work, too, contributed to direct attention to exploring the question on expression of lectins in vertebrates, especially in mammals. That a mammalian hepatic lectin proved to be responsible for rapid clearance of a glycoprotein (i.e. ceruloplasmin) from serum after loss of sialic acids from its N-glycans [18] and that a (β-)*ga*(lactose-binding)*lectin* from the electric eel (and from extracts of rat and chicken tissues and murine neuroblastoma cells) proved to be capable of bridging erythrocytes [19] established fundamental proof-of-principle evidence for the (patho)physiological relevance of this type of recognition [20,21]. 'Writing' glycan-encoded signals, being able to select and to 'read' distinct ‚signals' (glycans) from the wide variety and ‚translating' their messages into particular post-binding effects such as adhesion, cell-specific delivery (homing) or outside-in signaling are among the prerequisites to let „glycan functions pervade biology at all levels" [22], questions on molecular mechanisms arising. „One incentive for asking such questions [on glycans and transmission of signals] is their immediate relevance to medicine, in areas such as protection against infectious disease agents and for targeting of drugs and enzymes to sites where they would best achieve their therapeutic effects" [23]. In general terms, it appears that "glycans represent a largely untapped resource for biological discovery as well as unanticipated therapeutic opportunities" [24]. This aspect gives reason to explain interest in endogenous lectins, in our context in the family of the multifunctional galectins and its member classified as galectin-3 (Gal-3). The literature survey is based on routine monitoring of development of status of knowledge by Current Contents® combined with *Pubmed* searches and active research in the field for about 25 years or 35 years by the two authors.

2. Galectin-3: its detection and characterization

This galectin has independently been detected by immunological and biochemical lines of research. With the intention to generate specific reagents for immunocyto- and histochemical applications and for functional assays, cells or subcellular fractions have been and still are used as antigen to raise monoclonal antibodies. Panel testing of the resulting hybridoma clones can then lead to interesting products. This was the case in a project on characterizing

phenotypic divergence of mononuclear phagocytes by antibodies, especially stages of activation of peritoneal exudate phagocytes after injection of known elicitors into (C57BL/6) mice, especially thioglycollate. When rat spleen cells were immunized to nuclei-free extracts of cell preparations that had been depleted of previously identified antigens by monoclonal antibody immunoadsorbents, a new macrophage (Mac) antigen was defined: supernatants of the hybridoma clones M3/31 and M3/38 precipitated a 32 kDa protein from extracts of activated murine peritoneal macrophages, called the Mac-2 antigen [25,26]. By the way, the Mac-1 antigen that was first identified by this approach is a subunit of an integrin now known as CD11b (the α_M-integrin subunit, also called CR3) [27,28].

Independently, by using extracts of the murine B16-F1 melanoma cell line for immunization, a monoclonal antibody (clone 5D7) was obtained that blocked haemagglutination by cell extracts, bound to bands at 34 kDa (called L-34) and 68 kDa of purified agglutinin, showed cross-reactivity to β-galactoside-binding lectins from electric eel and adult chicken muscle and localized antigen on the surface and in the cytoplasm of murine (B16-F1 and K-1735 melanoma; UV-2237 fibrosarcoma) and human (Hs939 melanoma; SK-DZ neuroblastoma; HeLa-53 carcinoma) tumor cells [29].

The second approach to detecting the galectin took advantage of its selective binding property for β-galactosides. Inspired by the pioneering purification of electrolectin, the galectin from the electric eel [19] (which turned out to be the first galectin [30]), affinity chromatography with resins presenting β-galactosides (the two glycoproteins asialofetuin and immunoglobulin E (IgE) or lactose) was performed with extracts of different sources. A protein of a molecular weight between 31-35 kDa was found in eluted fractions, from extracts of fibroblasts (3T3 and SL66 cells, called carbohydrate-binding protein (CBP) 35) [31], of murine or human tumors [32,33], of *Xenopus* oocytes after *in vitro* translation directed by injected mRNA of rat basophilic leukemia cells (binding IgE, thus called εBP [34] and of rat lung (RL-29) [35]. Antibodies against purified protein revealed a broad profile of tissue expression in normal and tumor cells [36-38], also adding nuclear staining (apparently correlated to state of proliferation in 3T3 fibroblasts) to the sites of intracellular localization *in vitro* [39]. Biochemical and immunological comparisons as well as cloning and sequencing of the cDNAs converged to reveal that the Mac-2 (and 5D7) antigen, CBP35 (CBP30 in baby hamster kidney cells or RL-29) and εBP are all homologues of a particular gene present in mammals, i.e. the gene coding for a protein of a growing family of tissue lectins, referred to as galectin (for review, please see [40]).

This special protein's structure is composed of three types of modules (Figure 1). Its sequence starts with an N-terminal section containing two sites for serine phosphorylation, followed by a Tyr-, Pro- and Gly-rich non-triple helical collagen-like repeat region of nine amino acids per unit (these two parts comprise the N-terminal tail (NT)) and the carbohydrate recognition domain (CRD) (Figure 1). Its trimodular design explains the term chimera-type galectin, in relation to the mono- and homodimeric (proto type) and linker-connected heterodimeric (tandem-repeat type) members of the galectin family (Figure 1) [41-43].

As common properties, these proteins share the β-sandwich fold and the contact profile to the canonical ligand lactose via hydrogen bonding and C-H/π interactions. Key amino acid residues that form the conserved sequence signature with a central Trp residue (furnishing the π-electrons for contact to the three C-H sites on the B-face of D-galactose), are illustrated for CRDs of vertebrate galectins in Figure 2 (for further informations on crystallographic structures, please see [44]). Moreover, they all have no signal sequence so that galectins are secreted by not yet completely elucidated non-conventional routes [45]. As effectors, they are known to be multifunctional, active in intracellular compartments, on the cell surface and extracellularly, and opposite effects on cells (for example blocking or mediating adhesion or proliferation) depending on cell type, concentration and context are known [43,46-49]. In the words of K.-i. Kasai, "the same galectin species seems to participate in development, differentiation, morphogenesis, immunity, apoptosis, metastasis of malignant cells etc.... The biological function of galectins should vary from site to site, and from time to time.... The "one galectin – one phenomenon" relationship does not exist and the lack of specific and principal roles is the nature of the galectin family" [50]. In the classification system of the galectin family by numbers, the protein detected by the approaches given above was given the name Gal-3.

The structurally distinctive feature of Gal-3 with its unique trimodular design accounts for differences in the biological behavior. Among galectins, it opens the way toward special versatility in aggregate formation. Monomeric in solution, this galectin can form cross-linked complexes with glycoconjugate counterreceptors of characteristic topological order. They are different from lattices formed by a proto-type galectin, as revealed by precipitation and negative stain electron microscopy using a nonavalent glycoprotein (asialofetuin with its three complex-type N-glycans) and synthetic glycoclusters [51]. The combination of the collagen-like repeat section with the CRD, both capable to mediate self-aggregation (for recent literature surveys, please see [52,53]), underlies the difference relative to homodimeric Gal-1 that is solely built by one type of module, i.e. the CRD (Figure 1). As suggested for hamster

Gal-3 by applying NMR-spectroscopical analysis of full-length protein and fragments thereof as well as electron microscopical monitoring of Gal-3 preparations, "oligomerization of substratum-adsorbed galectin-3, through N- and C-terminal domain interactions, could be relevant to the positive cooperativity observed in binding of the lectin to immobilized multiglycosylated proteins such as laminin" [54]. Of note, the nature of the ligand is assumed to affect the aggregation process in a special manner [52,55-57]. On the atomic level, crystallographic mapping of intermolecular CRD contacts has recently become available (Figure 3) [53].

In comparison to the CRD, the enormous flexibility of the NT with its collagen-like repeats is precluding to reach the same level of structural analysis. The physiological truncation of this section by proteases, especially matrix metalloproteinases, that modulates Gal-3's bioactivity, as shown e.g. in breast cancer cells [58] or neutrophils [59], has inspired design of Gal-3 variants by stepwise deletion of sequence repeats. This approach is instrumental to explore the significance of number of tandem repeats and to reduce extent of flexibility [60]. Indeed, first crystallographic information on a part of the NT has become available by using a variant generated this way (Figure 4) [53]. Of interest in this context, the CRD, too, has inherent spatial flexibility. When binding the ligand lactose, its accommodation, is associated with an increase in conformational entropy of the protein without any structural changes, a substantial contribution to the thermodynamics and a caveat to solely base drug-design efforts on static X-ray structures [61].

In further structural terms, the nature of galectins as homologous proteins (for a phylogenetic tree of this family, please see [62]) with conservation of the signature sequence and its presentation in the binding site, as shown in Figure 2, let it expect that their binding profiles to glycans are rather similar, *cum grano salis*. Assumed similarity is for example reflected in the compilation of respective results obtained by frontal affinity chromatography [63]. Substitutions of the canonical N-acetyllactosamine (LacNAc) core to build blood group ABH(0) epitopes therefore are favorable to increase affinity not only for human Gal-3, as initially detected by inhibition assays using glycans to interfere with galectin-asialofetuin (immobilized on Sepharose beads) interaction [64], but also for the tandem-repeat-type galectins-4, -8 and -9 [63]. That occurrence of 'cross-reactivity' for glycans among galectins is naturally common is further exemplified for tandem-repeat-type galectins-4 and -9. They interact with a Gal-3-binding TF-disaccharide-presenting glycopeptide at low nM-affinity [65], as 3'-sulfated lactose is a contact site for glycodendrimersome bridging for these galectins [66]. Adding an example for proto-type proteins, galectins-2 and -7 bridge

nanoparticles with the parasitic signature disaccharide LacdiNAc (GalNAcβ1,4GlcNAc), a contact point first reported for Gal-3 and assumed to be specific due to the reported lack of binding by Gal-1 [67,68]. Such case studies advise rigorous comprehensive panel testing prior to claiming inter-galectin specificity.

On the level of the natural glycoconjugate counterreceptors, multivalency of glycoproteins, clustering in microdomains and the evidence for a gradient of decreasing affinity constants upon loading with galectin make "fractional high-affinity binding" and thus "supramolecular assemblies" already at low galectin concentrations possible that "can trigger cell surface signal transduction mechanism" [69]. To do so, the galectin selects few binding partners for building aggregates, and this – as far as we know at this moment – depending on cell type (for a compilation of glycoconjugate counterreceptors, please see Table 1). Of note, the level of expression of the protein as scaffold for glycans and the structure of the glycan part, especially the status of α2,6-sialylation of N-glycans, are effective regulators of extent of galectin binding. In addition, "a majority of Gal-3's intracellular interactions occur via protein-protein interactions" [70]: respective binding partners are also presented in Table 1. Except for the case of Gal-3–B cell lymphoma 2 (Bcl-2) recognition that appears to involve the NWGR motif also essential for lactose binding and thus is impaired by presence of the cognate sugar [71,72], the atomic details on Gal-3–protein interactions await structural characterization.

In summary, whereas the analysis of the structure and binding properties to glycans of the CRD of Gal-3 revealed (expectable) similarities, the trimodular design is unique (Figure 1). The currently described panel of counterreceptors, given in Table 1, substantiates the validity of the assumption for a selective process, as implied by the term 'lectin'. The current status of insight into this galectin's functional profile invites to deliberate pros and cons of considering Gal-3 as a therapeutic target.

4. Gal-3: a therapeutic target?

In principle, three fundamental points deserve attention to answer this question:

1. As already noted above, galectins are multifunctional, and the literature provides ample evidence to encourage further investigations. Thus, with focus on tumor biology, "it is increasingly recognized that galectin-3 is an important regulator of a broad range of cancer cell activities and plays important roles in cancer cell growth, transformation, apoptosis, angiogenesis, adhesion, invasion and metastasis. Such a divergent influence of galectin-3 on cancer activities derives from its multiple inter-

and sub-cellular localizations where it interacts with a range of different binding partners" [73]. A recent study on negative correlation between Gal-3 presence and β_3-integrin expression, pointing to nuclear events on the level of the promoter, in murine melanoma metastasis concluded that "it is possible that even a single molecule can affect several different processes of cancer cell biology in a context-dependent manner" [74]. Due to its wide range of expression, adding to its multifunctionality, short- and long-term consequences of any kind of targeting Gal-3 systemically will definitely need to be examined thoroughly.

2. Evidence for an involvement of Gal-3 in pathogenesis is often derived from work with mouse models deficient in galectin expression, as exemplified by the following statement: "based on these data [on galectin-3 null mice], it appears that galectin-3 protein is implicated in the development of fibrosis resulting from inflammatory or toxic insults, thereby establishing a rationale to antagonize its function to treat fibrosis" [75]. Experience with such a model in tumor biology taught a salient lesson deserving to be noticed: the (apparently unexpected) lack of impact of absence of Gal-3 in mouse models genetically programmed to develop cancer "could possibly be explained by the fact that adaptive mechanisms arise" [76]. Such alterations as response to a knock-out may not only be instrumental for maintaining homeostasis, as documented *in vivo* upon creating a deficiency in N-glycan branching, a factor relevant for binding of tissue lectins [77-79]. They may also cause not readily predictable outcomes, calling for cautious interpretation. Of course, and nearly needless to say, general extrapolation from mice to man, too, is less than straightforward. Looking at the equipment with lectins between species on the genome level, pronounced differences of not yet fully understood consequences have been detected, for example in the case of a C-type lectin (DC-SIGN, CD209) with eight genetic homologs in mice and no clear ortholog of the human protein [80], and also the genetic background can affect characteristics of the loss-of-function phenotype, as discussed for Gal-1 [81].
3. While exploring the expression of the members of the family of galectins step by step and performing functional analysis on each protein, the emerging aim is to elucidate the possibility for interplay between galectins. Fittingly, initial comprehensive network analysis for galectins by immunohistochemistry revealed expression profiles with overlaps and differences [82]. Galectins may therefore influence each other's activities when being co-expressed, and this context dependently in different manners.

In fact, pilot studies have disclosed that i) Gal-3 acts as an antagonist of Gal-1 in growth regulation [83,84], ii) it can trigger apoptosis in primary activated T cells in contrast to Gal-1, which in neutrophils yet both induce phosphatidylserine exposure [85] and iii) it can cooperate with Gal-1 and -8 in osteoarthritis pathogenesis [86]. These initial insights into versatility of functional interplay offer opportunities and challenges.

In addition to these issues, any targeting approach will need to take similarities among the members of this mammalian family in protein structure and in ligand binding, here also for other types of lectins with affinity to LacNAc and its derivatives, into consideration. As listed in the following paragraph, several approaches are in principle capable to interfere with Gal-3 activities:

1. The available monoclonal antibodies, especially with epitope recognition in the tail, appear to allow blocking of extracellular Gal-3. Of note, presence of autoantibodies against Gal-3 in human serum [87] should be taken into account when analyzing data on Gal-3 obtained with clinical samples, as is also the case for galectin presence in serum and cellular microenvironments.
2. Small molecule inhibitors, especially derivatized lactose or thiodigalactoside, and natural polysaccharides such as processed citrus pectin are possible means to interfere with carbohydrate-dependent binding, (most probably) mostly extracellularly, as antibodies can do. Their target selectivity and specificity as well as potential to trigger effects via off-target binding is currently under dispute for polysaccharides [88,89]. Design of glycoclusters suited to distinguish of different modular architecture (shown in Figure 1), in analogy to work on the hepatic asialoglycoprotein receptor that established the concept of the glycoside cluster effect [90], has potential to exploit difference in protein architecture (Gal-3 vs Gal-1; [91]).
3. As a means to downregulate Gal-3 presence globally, also in nuclei and cytoplasm, siRNA offers the potential to switch off production after cell-type-specific delivery. Rigorous controls for off-target effects, determination of extent of achievable silencing and analysis on occurrence of any “adaptive mechanisms” are mandatory.
4. Viewed from the counterreceptor side, Gal-3-driven pathogenesis could ideally be slowed by manipulations on glycan (or cognate protein; for examples, please see bottom part of Table 1) accessibility or presence. Masking of Gal-3-binding

glycans, using as platform for amendments Gal-3-derived ligand-binding peptides [92], or downregulating distinct counterreceptors (cognate glycosylation and/or its protein scaffold; cognate proteins) are means to lower output of Gal-3-dependent signaling.

5. An emerging strategy is to direct Gal-3-dependent signaling *in situ* into a favorable direction by letting a Gal-3 variant with a new type of architecture act as competitor and elicitor. Proof of principle to this concept has recently been obtained by demonstrating an engineered Gal-3 homodimer that exhibits anti-proliferative activity for human neuroblastoma (SK-N-MC) cells so that endogenous (chimera-type) Gal-3 is more than simply neutralized [68]. Summarizing its effects, this variant should compete with the wild-type protein for binding to counterreceptors (neutralization) and initiate different (at best opposite) post-binding signaling (elicitor activity) due to changes in the topology of formed lattices. Obviously, such variants can prove very useful in delineating the relationship between galectin architecture and lattice structure.
   In essence, a series of hurdles has to be cleared to reach the aim to define Gal-3 as a therapeutic target, as outlined in the first part of this section. When this has successfully been done, then work on an approach to evaluate the possibility for blocking may follow. In the case of a multifunctional protein acting at many sites and this likely in a network, this is not a trivial task.

5. Conclusion

Guided by the assumptions that "complex carbohydrate-containing molecules may function in synaptic recognition and transmission through establishment of cell-cell contacts and possibly as mediators of communication between the surface and the interior of the cell" and – "in line with these ideas" – and that therefore presence of "protein capable of interacting with saccharides" is expectable, the first galectin had been detected by its β-galactoside-inhibitable bridging of trypsin-treated rabbit erythrocytes and purified by affinity chromatography [19]. This work was the starting point for discovering a family of proteins that share affinity to the canonical ligand lactose and the β-sandwich fold. They are present in many types of cells, on the cellular level intracellularly and after non-conventional secretion in the medium or bound to the cell surface and in the matrix. Structurally, their modular design guides to classify vertebrate galectins into the three classifications presented in Figure 1. The common CRD has the expectable similarity among members of the family illustrated in Figure 2.

On the level of their cellular binding partners, galectins appear to be highly selective for certain binding partners. Faced with the large variety of cellular glycoconjugates, they engage in functional pairing with few distinct counterreceptors. Their suitability to associate to galectin(s) is dynamically regulated (levels of protein or sphingolipid presence and of cognate glycosylation). In addition, peptide motifs satisfy criteria for recognition (for examples, please see Table 1).

Since the discovery of galectins, special attention has been given to the investigation of galectins-1 and -3. In addition to further broadening our knowledge on these two proteins and to systematically filling gaps by studying all family members, the investigation of their activity profiles in mixtures, mimicking the *in vivo* situation, is an emerging challenge. Since simple extrapolations from *in vitro* models to the *in vivo* situation and from mouse models to man are not valid, progress toward defining clinical situation(s) as candidates for considering galectin-based interventions is expected to be slow, as has been observed in the case of the selectins. In this field, the recent testing of a small molecule inhibitor and a humanized anti-P-selectin monoclonal antibody revealed potential in reducing time or rate of sickle cell-related vaso-occlusive pain crises [93-95]. *Mutatis mutandis*, and selectins have a much narrower functionality profile as cell adhesion molecules than galectins, problems and considerations how to proceed are similar: “given the inherent risks in extrapolating data from animal models to humans, careful selection of clinical targets and proper trial design will be essential for further progress” [93].

6. Expert opinion

Research on galectins is a highly fertile field that is contributing exciting lines of evidence to substantiate the validity of the concept of the sugar code. Having started with the analysis of individual members of the family, an emerging topic of research is the network monitoring. The growing awareness of the possibility of local co-presence sets the stage for the discovery of functional interplay even including antagonism.

Of equal importance, it is becoming apparent that availability of counterreceptors for galectin(s) is intimately controlled to gain specific effects at distinct places and time points. The galectins’ ability to recognize β-galactosides or glycosaminoglycans or sphingolipids (from 3’-O-sulfated galactose to oligosaccharides) enables their broad-scale involvement in the flow of biological information in (patho)physiological processes. The amazingly fine-tuned coordination of glycosylation (i.e. sialylation via throttling sialic acid biosynthesis) with galectin, and counterreceptor (i.e. $\alpha_5\beta_1$-integrin) expression by a tumor suppressor, i.e.

$p16^{INK4a}$, to facilitate susceptibility of pancreatic carcinoma (Capan-1) cells to anoikis induction by Gal-1 *in vitro* [96] is one of several examples pointing to potential for (patho)physiological significance, if these changes will be documented to occur *in vivo*. Essentially, the indicated cross-talk between galectins (and probably other types of lectins) as well as between galectins and the glycosylation/glycoconjugate profile needs to be resolved, then set into relation to distinct disease processes *in vitro* and *in vivo*. Multifunctionality and multi-site expression of the galectins, their likely interplay at sites of co-expression and the ~~emerging~~ noted variability of counterreceptors on the levels of glycan and scaffold structures among cell types including dynamic regulatory mechanisms (up- and downregulation of *de novo* synthesis and enzymatic remodeling of glycans) make galectins demanding but fascinating study objects. Admittedly, a clinical impact is rather likely not just around the corner.

In general, it is not uncommon that, as encountered as factor to explain the low success rate in phase III randomized clinical trials (RCTs for cancer), "investigators consistently make overly-optimistic assumptions regarding treatment benefits when designing RCTs" [97]. Following a 4-step validation procedure patiently and perseveringly can help decrease likelihood of disappointments [98]. The status of clinical testing in galectin-related phase I/II trials has recently been reviewed with the conclusion that "some of these strategies will be more successful than others" [99]. As stated in connection with the just mentioned assessment of failure rate in RCTs in oncology, "although our goals may initially be lofty, they eventually meet reality" [100]. In this spirit, the aim of reaching an understanding of context-dependent functionality of these multi-purpose effectors alone and in the natural network, and this in models of reasonable translatability, will most likely keep us busy for time to come.

Funding

This paper was not funded.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose

References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers

1. Boyd WC, Shapleigh E. Specific precipitating activity of plant agglutinins (lectins). Science 1954;119:419
2. Boyd WC. The lectins: their present status. Vox Sang 1963;8:1-32
3. Kocourek J. Historical background. In: Liener IE, Sharon N, Goldstein IJ, eds. The Lectins. Properties, functions and applications in biology and medicine. New York: Academic Press 1986:1-32
4. Kaltner H, García Caballero G, Ludwig A-K, et al. From glycophenotyping by (plant) lectin histochemistry to defining functionality of glycans by pairing with endogenous lectins. Histochem Cell Biol 2018;149:547-68
5. Laine RA. The information-storing potential of the sugar code. In: Gabius H-J, Gabius S, eds. Glycosciences: Status and Perspectives. London - Weinheim: Chapman & Hall 1997:1-14
6. Sharon N. Complex Carbohydrates. Their chemistry, biosynthesis, and functions. Reading, MA, USA: Addison-Wesley Publ. Co., 1975
7. Ginsburg V, Neufeld EF. Complex heterosaccharides of animals. Annu Rev Biochem 1969;38:371-88
8. Roseman S. Reflections on glycobiology. J Biol Chem 2001;276:41527-42
9. Corfield AP. Eukaryotic protein glycosylation: a primer for histochemists and cell biologists. Histochem Cell Biol 2017;147:119-47
10. Kopitz J. Lipid glycosylation: a primer for histochemists and cell biologists. Histochem Cell Biol 2017;147:175-98
11. Gabius H-J, ed. The Sugar Code. Fundamentals of glycosciences. Weinheim, Germany: Wiley-VCH 2009
12. Gabius H-J, Roth J. An introduction to the sugar code. Histochem Cell Biol 2017;147:111-7
13. Reuter G, Gabius H-J. Eukaryotic glycosylation: whim of nature or multipurpose tool? Cell Mol Life Sci 1999;55:368-422
14. Togayachi A, Narimatsu H. Functional analysis of β1,3-N-acetylglucosaminyltransferases and regulation of immunological function by polylactosamine. Trends Glycosci Glycotechnol 2012;24:95-111

15. Manning JC, Romero A, Habermann FA, et al. Lectins: a primer for histochemists and cell biologists. Histochem Cell Biol 2017;147:199-222
16. Kaltner H, Abad-Rodríguez J, Corfield AP, et al. The sugar code: letters and vocabulary, writers, editors and readers and biosignificance of functional glycan-lectin pairing. Biochem J 2019;476:2623-55
17. Roth J. Lectins for histochemical demonstration of glycans. Histochem Cell Biol 2011;136:117-30
18. Hudgin RL, Pricer WEJ, Ashwell G, et al. The isolation and properties of a rabbit liver binding protein specific for asialoglycoproteins. J Biol Chem 1974;249:5536-43
19. Teichberg VI, Silman I, Beitsch DD, Resheff G. A β-D-galactoside binding protein from electric organ tissue of *Electrophorus electricus*. Proc Natl Acad Sci USA 1975;72:1383-7
•• **Pioneering study on detection of lectin activity in extracts of embryonic and tumor cells, the need to add reducing agent for protection of lectin activity and the first purification of a galectin.**
20. Barondes SH. Lectins: their multiple endogenous cellular functions. Annu Rev Biochem 1981;50:207-31
21. Gabius H-J. Animal lectins. Eur J Biochem 1997;243:543-76
22. Hart GW. Thematic minireview series on glycobiology and extracellular matrices: glycan functions pervade biology at all levels. J Biol Chem 2013;288:6903
23. Hughes RC, Sharon A. Carbohydrates recognised. Nature 1978;274:637-8
24. Agre P, Bertozzi C, Bissell M, et al. Training the next generation of biomedical investigators in glycosciences. J Clin Invest 2016;126:405-8
25. Springer TA. Monoclonal antibody analysis of complex biological systems. Combination of cell hybridization and immunoadsorbents in a novel cascade procedure and its application to the macrophage cell surface. J Biol Chem 1981;256:3833-9
26. Ho MK, Springer TA. Mac-2, a novel 32,000 M_r mouse macrophage subpopulation-specific antigen defined by monoclonal antibodies. J Immunol 1982;128:1221-8
27. Springer T, Galfre G, Secher DS, Milstein C. Monoclonal xenogeneic antibodies to murine cell surface antigens: identification of novel leukocyte differentiation antigens. Eur J Immunol 1978;8:539-51
28. Springer T, Galfre G, Secher DS, Milstein C. Mac-1: a macrophage differentiation antigen identified by monoclonal antibody. Eur J Immunol 1979;9:301-6

29. Raz A, Meromsky L, Carmi P, et al. Monoclonal antibodies to endogenous galactose-specific tumor cell lectins. EMBO J 1984;3:2979-83
30. Barondes SH. Galectins: a personal overview. Trends Glycosci Glycotechnol 1997;9:1-7
- **A very-worth-to-read personal essay on the origin of the galectin field by one of its pioneers.**
31. Roff CF, Wang JL. Endogenous lectins from cultured cells. Isolation and characterization of carbohydrate-binding proteins from 3T3 fibroblasts. J Biol Chem 1983;258:10657-63
32. Gabius H-J, Engelhardt R, Rehm S, Cramer F. Biochemical characterization of endogenous carbohydrate-binding proteins from spontaneous murine rhabdomyosarcoma, mammary adenocarcinoma, and ovarian teratoma. J Natl Cancer Inst 1984;73:1349-57
33. Gabius H-J, Engelhardt R, Cramer F, et al. Pattern of endogenous lectins in a human epithelial tumor. Cancer Res 1985;45:253-7
34. Liu FT, Orida N. Synthesis of surface immunoglobulin E receptor in *Xenopus* oocytes by translation of mRNA from rat basophilic leukemia cells. J Biol Chem 1984;259:10649-52
35. Cerra RF, Gitt MA, Barondes SH. Three soluble rat β-galactoside-binding lectins. J Biol Chem 1985;260:10474-7
36. Crittenden SL, Roff CF, Wang JL. Carbohydrate-binding protein 35: identification of the galactose-specific lectin in various tissues of mice. Mol Cell Biol 1984;4:1252-9
37. Gabius H-J, Brehler R, Schauer A, Cramer F. Localization of endogenous lectins in normal human breast, benign breast lesions and mammary carcinomas. Virch Arch [Cell Pathol] 1986;52:107-15
38. Gritzmacher CA, Robertson MW, Liu FT. IgE-binding protein. Subcellular location and gene expression in many murine tissues and cells. J Immunol 1988;141:2801-6
39. Moutsatsos IK, Wade M, Schindler M, Wang JL. Endogenous lectins from cultured cells: nuclear localization of carbohydrate-binding protein 35 in proliferating 3T3 fibroblasts. Proc Natl Acad Sci USA 1987;84:6452-6
40. Hughes RC. Mac-2: a versatile galactose-binding protein of mammalian tissues. Glycobiology 1994;4:5-12
41. Kasai K-i, Hirabayashi J. Galectins: a family of animal lectins that decipher glycocodes. J Biochem 1996;119:1-8

42. Cooper DNW. Galectinomics: finding themes in complexity. Biochim Biophys Acta 2002;1572:209-31
43. Kaltner H, Toegel S, García Caballero G, et al. Galectins: their network and roles in immunity/tumor growth control. Histochem Cell Biol 2017;147:239-56
44. Kamitori S. Three-dimensional structures of galectins. Trends Glycosci Glycotechnol 2018;30:SE41-SE50
45. Hughes RC. Secretion of the galectin family of mammalian carbohydrate-binding proteins. Biochim Biophys Acta 1999;1473:172-85
46. Hirabayashi J, ed. Recent topics on galectins. Trends Glycosci Glycotechnol 1997;9:1-180 and Special issue on galectins. Trends Glycosci Glycotechnol 2018;30:SE1-SE223
•• **What-you-need-to-know collections of reviews on galectins.**
47. Liu F-T, Patterson RJ, Wang JL. Intracellular functions of galectins. Biochim Biophys Acta 2002;1572:263-73
48. Kasai K-i. Galectins: quadruple-faced proteins. Trends Glycosci Glycotechnol 2018;30:SE221-SE3
49. Sato S. Cytosolic galectins and their release and roles as carbohydrate-binding proteins in host-pathogen interaction. Trends Glycosci Glycotechnol 2018;30:SE199-SE209
50. Kasai K-i. Galectin: intelligent glue, non-bureaucratic bureaucrat or almighty supporting actor. Trends Glycosci Glycotechnol 1997;9:167-70
51. Ahmad N, Gabius H-J, André S, et al. Galectin-3 precipitates as a pentamer with synthetic multivalent carbohydrates and forms heterogeneous cross-linked complexes. J Biol Chem 2004;279:10841-7
52. Lin YH, Qiu DC, Chang WH, et al. The intrinsically disordered N-terminal domain of galectin-3 dynamically mediates multisite self-association of the protein through fuzzy interactions. J Biol Chem 2017;292:17845-56
53. Flores-Ibarra A, Vértesy S, Medrano FJ, et al. Crystallization of a human galectin-3 variant with two ordered segments in the shortened N-terminal tail. Sci Rep 2018;8:9835
54. Birdsall B, Feeney J, Burdett ID, et al. NMR solution studies of hamster galectin-3 and electron microscopic visualization of surface-adsorbed complexes: evidence for interactions between the N- and C-terminal domains. Biochemistry 2001;40:4859-66
55. Lepur A, Salomonsson E, Nilsson UJ, et al. Ligand induced galectin-3 protein self-association. J Biol Chem 2012;287:21751-6

56. Halimi H, Rigato A, Byrne D, et al. Glycan dependence of galectin-3 self-association properties. PLoS One 2014;9:e111836
57. Ippel H, Miller MC, Vértesy S, et al. Intra- and intermolecular interactions of human galectin-3: assessment by full-assignment-based NMR. Glycobiology 2016;26:888-903
58. Nangia-Makker P, Wang Y, Raz T, et al. Cleavage of galectin-3 by matrix metalloproteases induces angiogenesis in breast cancer. Int J Cancer 2010;127:2530-41
59. Sundqvist M, Welin A, Elmwall J, et al. Galectin-3 type-C self-association on neutrophil surfaces: the carbohydrate recognition domain regulates cell function. J Leukoc Biol 2018;103:341-53
60. Kopitz J, Vértesy S, André S, et al. Human chimera-type galectin-3: defining the critical tail length for high-affinity glycoprotein/cell surface binding and functional competition with galectin-1 in neuroblastoma cell growth regulation. Biochimie 2014;104:90-9
61. Verteramo ML, Stenstrom O, Ignjatovic MM, et al. Interplay between conformational entropy and solvation entropy in protein-ligand binding. J Am Chem Soc 2019;141:2012-26
62. Houzelstein D, Gonçalves IR, Fadden AJ, et al. Phylogenetic analysis of the vertebrate galectin family. Mol Biol Evol 2004;21:1177-87
63. Iwaki J, Hirabayashi J. Carbohydrate-binding specificity of human galectins: an overview by frontal affinity chromatography. Trends Glycosci Glycotechnol 2018;30:SE137-SE53
- **Survey of glycan binding for human galectins that illustrates 'cross-reactivity' among the members of the family, a caveat for too high expectations on target selectivity of small molecule inhibitors.**
64. Sparrow C, Leffler H, Barondes SH. Multiple soluble β-galactoside-binding lectins from human lung. J Biol Chem 1987;262:7383-90
65. Guha P, Kaptan E, Bandyopadhyaya G, et al. Cod glycopeptide with picomolar affinity to galectin-3 suppresses T-cell apoptosis and prostate cancer metastasis. Proc Natl Acad Sci USA 2013;110:5052-7
66. Xiao Q, Ludwig A-K, Romano C, et al. Exploring functional pairing between surface glycoconjugates and human galectins using programmable glycodendrimersomes. Proc Natl Acad Sci USA 2018;115:E2509-E18
67. van den Berg TK, Honing H, Franke N, et al. LacdiNAc-glycans constitute a parasite pattern for galectin-3-mediated immune recognition. J Immunol 2004;173:1902-7

68. Ludwig A-K, Michalak M, Xiao Q, et al. Design-functionality relationships for adhesion/growth-regulatory galectins. Proc Natl Acad Sci USA 2019;116:2837-42

•• **Delineation of functional impact of altering modular architecture by testing CRD-sharing proteins with various types of design in aggregation (using bottom-up chemical tailoring of nanoparticle surface) and proliferation assays.**

69. Dam TK, Gabius H-J, André S, et al. Galectins bind to the multivalent glycoprotein asialofetuin with enhanced affinities and a gradient of decreasing binding constants. Biochemistry 2005;44:12564-71

70. Funasaka T, Raz A, Nangia-Makker P. Nuclear transport of galectin-3 and its therapeutic implications. Semin Cancer Biol 2014;27:30-8

71. Yang R-Y, Hsu DK, Liu F-T. Expression of galectin-3 modulates T-cell growth and apoptosis. Proc Natl Acad Sci USA 1996;93:6737-42

72. Harazono Y, Nakajima K, Raz A. Why anti-Bcl-2 clinical trials fail: a solution. Cancer Metast Rev 2014;33:285-94

• **An inspiring literature survey that highlights the essential nature of a holistic consideration on networking between protein families for trial design.**

73. Newlaczyl AU, Yu LG. Galectin-3: a jack-of-all-trades in cancer. Cancer Lett 2011;313:123-8

74. Hayashi Y, Jia W, Kidoya H, et al. Galectin-3 inhibits cancer metastasis by negatively regulating integrin β3 expression. Am J Pathol 2019;189:900-10

75. Traber PG, Chou H, Zomer E, et al. Regression of fibrosis and reversal of cirrhosis in rats by galectin inhibitors in thioacetamide-induced liver disease. PLoS One 2013;8:e75361

76. Eude-Le Parco I, Gendronneau G, Dang T, et al. Genetic assessment of the importance of galectin-3 in cancer initiation, progression, and dissemination in mice. Glycobiology 2009;19:68-75

77. Dam TK, Brewer CF. Maintenance of cell surface glycan density by lectin-glycan interactions: a homeostatic and innate immune regulatory mechanism. Glycobiology 2010;20:1061-4

78. Ismail MN, Stone EL, Panico M, et al. High-sensitivity O-glycomic analysis of mice deficient in core 2 β1,6-N-acetylglucosaminyltransferases. Glycobiology 2011;21:82-98

79. Ohtsubo K. Biological significance of N-acetylglucosaminyltransferase-IV-mediated protein glycosylation on the homeostasis of cellular physiological functions. Trends Glycosci Glycotechnol 2011;23:103-5

80. Garcia-Vallejo JJ, van Kooyk Y. The physiological role of DC-SIGN: a tale of mice and men. Trends Immunol 2013;34:482-6
81. Sakaguchi M, Okano H. Neural stem cells, adult neurogenesis, and galectin-1: from bench to bedside. Developmental neurobiology 2012;72:1059-67
82. Manning JC, García Caballero G, Knospe C, et al. Network analysis of adhesion/growth-regulatory galectins and their binding sites in adult chicken retina and choroid. J Anat 2017;231:23-37
83. Kopitz J, von Reitzenstein C, André S, et al. Negative regulation of neuroblastoma cell growth by carbohydrate-dependent surface binding of galectin-1 and functional divergence from galectin-3. J Biol Chem 2001;276:35917-23
84. Sanchez-Ruderisch H, Fischer C, Detjen KM, et al. Tumor suppressor $p16^{INK4a}$: downregulation of galectin-3, an endogenous competitor of the pro-anoikis effector galectin-1, in a pancreatic carcinoma model. FEBS J 2010;277:3552-63
85. Stowell SR, Qian Y, Karmakar S, et al. Differential roles of galectin-1 and galectin-3 in regulating leukocyte viability and cytokine secretion. J Immunol 2008;180:3091-102
86. Weinmann D, Kenn M, Schmidt S, et al. Galectin-8 induces functional disease markers in human osteoarthritis and cooperates with galectins-1 and -3. Cell Mol Life Sci 2018;75:4187-205
87. Mathews KP, Konstantinov KN, Kuwabara I, et al. Evidence for IgG autoantibodies to galectin-3, a β-galactoside-binding lectin (Mac-2, ε binding protein, or carbohydrate binding protein 35), in human serum. J Clin Immunol 1995;15:329-37
88. Nangia-Makker P, Hogan V, Honjo Y, et al. Inhibition of human cancer cell growth and metastasis in nude mice by oral intake of modified citrus pectin. J Natl Cancer Inst 2002;94:1854-62
89. Stegmayr J, Lepur A, Kahl-Knutson B, et al. Low or no inhibitory potency of the canonical galectin carbohydrate-binding site by pectins and galactomannans. J Biol Chem 2016;291:13318-34
90. Lee YC. Biochemistry of carbohydrate-protein interaction. FASEB J 1992;6:3193-200.
91. André S, Grandjean C, Gautier FM, et al. Combining carbohydrate substitutions at bioinspired positions with multivalent presentation towards optimising lectin inhibitors: case study with calixarenes. Chem Commun 2011;47:6126-8
92. Moise A, André S, Eggers F, et al. Toward bioinspired galectin mimetics: identification of ligand-contacting peptides by proteolytic-excision mass spectrometry. J Am Chem Soc 2011;133:14844-7

93. McEver RP. Selectins: initiators of leucocyte adhesion and signalling at the vascular wall. Cardiovasc Res 2015;107:331-9
94. Okpala I. Investigational selectin-targeted therapy of sickle cell disease. Expert Opin Investig Drugs 2015;24:229-38
95. Kutlar A, Kanter J, Liles DK, et al. Effect of crizanlizumab on pain crises in subgroups of patients with sickle cell disease: A SUSTAIN study analysis. Am J Hematol 2019;94:55-61
96. Amano M, Eriksson H, Manning JC, et al. Tumour suppressor $p16^{INK4a}$: anoikis-favouring decrease in N/O-glycan/cell surface sialylation by down-regulation of enzymes in sialic acid biosynthesis in tandem in a pancreatic carcinoma model. FEBS J 2012;279:4062-80
97. Gan HK, You B, Pond GR, Chen EX. Assumptions of expected benefits in randomized phase III trials evaluating systemic treatments for cancer. J Natl Cancer Inst 2012;104:590-8
•• **A knowledge- and experience-based encouragement for realistic assessment of collected data before considering to proceed to phase III clinical trial testing.**
98. Bunnage ME, Chekler EL, Jones LH. Target validation using chemical probes. Nat Chem Biol 2013;9:195-9
99. Girard A, Magnani J. Clinical trials and applications of galectin antagonists. Trends Glycosci Glycotechnol 2018;30:SE211-SE20
100. Amiri-Kordestani L, Fojo T. Why do phase III clinical trials in oncology fail so often? J Natl Cancer Inst 2012;104:568-9

List of abbreviations

CBP, carbohydrate-binding protein; CRD, carbohydrate recognition domain; Gal, (β-) ga(lactoside-binding)lectin = galectin; IgE/G, immunoglobulin E/G; Mac, macrophage; NT, N-terminal tail (of Gal-3); RCT, randomized clinical trial

Figure 1. Modular architecture of vertebrate galectins. The canonical carbohydrate recognition domain (CRD) is presented in three versions in vertebrates: a hybrid with collagen-like repeats (nine in man) and an N-terminal peptide with two sites of serine phosphorylation (top), a non-covalently associated homodimer (center) and a linker-connected dimer of two different domains (bottom).

Figure 2. Illustration of the structural homology and the canonical contact pattern in ligand binding based on the signature sequence of galectins. a: galectin-1 (Gal-1) (PDB code: 1GZW), b: Gal-2 (5DG2), c: Gal-3 (4LBN), d: Gal-4N (5DUW), e, f: Gal-4C with 3'-sulfated lactose (4YM2) or lacto-N-neotetraose (4YL7) as ligand, g: Gal-7 (4XBQ), h: Gal-8N (3AP9), i, j: Gal-9N with the Forssman pentasaccharide (2EAL) or the N-acetyllactosamine (LacNAc) dimer (DiLacNAc) (2ZHK) as ligand, k: Gal-9C (3NV2) and l: the chicken *ga*lectin-*r*elated *i*nter-*f*iber prote*in* (C-GRIFIN) (5NLD).

Figure 3. Crystal structure of a variant of human Gal-3 with CRD, collagen-like tandem repeats VII-IX (in purple) and the N-terminal section (in green; please see Figure 1 for modular design of Gal-3) in ribbon (left) and in surface presentation (right); for details, please see [53].

Figure 4. Illustration of CRD-CRD interactions in the case of the crystallized truncated Gal-3 version shown in Figure 3. Three tetramers face each other, each subunit colored. The highlighted central area is given below, showing regions involved in cross-contacts for each subunit (left) and a snapshot showing residues involved in cross-contacts between CRDs and lactose (right); for details, please see [53].

Table 1. Compilation of documented counterreceptors (glycan part of cellular glycoconjugates, proteins) of mammalian galectin-3

type of ligand	galectin-3
glycan	CD6, CD7, CD11b of CD11b/CD18 (Mac-1 antigen, CR3), CD13 (aminopeptidase N), CD32, CD43, CD44, CD45, CD66a,b, CD71, CD95, CD98, CD146 (MCAM, MUC18), CD147, CD166 (ALCAM), CEA, colon cancer mucin, corneal mucin (MUC16), pancreas cancer mucin-4 and MUC1-D (N-glycan at Asn36), cubilin, C4.4A (member of Ly6 family), mDectin-1/2, desmoglein-2, epidermal growth factor receptor, glycoform of IgE, haptoglobin β-subunit (after desialylation), hensin (DMBT-1), insulin receptor, insulin-like growth factor-1 receptor, β_1-integrin (CD29), $\alpha_4/\alpha_5/\beta_1$- and $\alpha_v\beta_3$-integrins, interferon-γ, keratan sulfate, LI-cadherin, laminin, lamp-1/-2, lubricin, Mac-2-binding protein, Mac-3, MAG, MP20 (tetraspanin), Na^+/K^+-ATPase, NG2 proteoglycan, NKp30, TCR complex, tenascin, tissue plasminogen activator, SIGN-R1, Toll-like receptor-4, transforming growth factor-β receptor, vascular cell adhesion molecule-1, vascular endothelial growth factor receptor 2, von Willebrand factor, ganglioside GM1
protein	AGE products, Alix/AIP-1, ATP synthase b-subunit, axin, bax, bcl-2, β-catenin, Cys/His-rich protein, Gemin4, glycogen synthase kinase-3β, hnRNP A2B1, hnRNP Q, mSufu, Mer receptor tyrosine kinase, myosin-2A, NLRP3 (NOD-like receptor family, pyrin domain containing 3, cryoporin), non-receptor tyrosine kinases c-Abl and Arg, nucleoporin Nup98, nucling, oncogenic K-Ras, OCA-B, pCIP, PIAS1, synexin (annexin VII), Tsg101, TTF-1, tripartite motif protein (TRIM) 16 (and also 5α, 6, 17, 20, 22, 23 and 49)

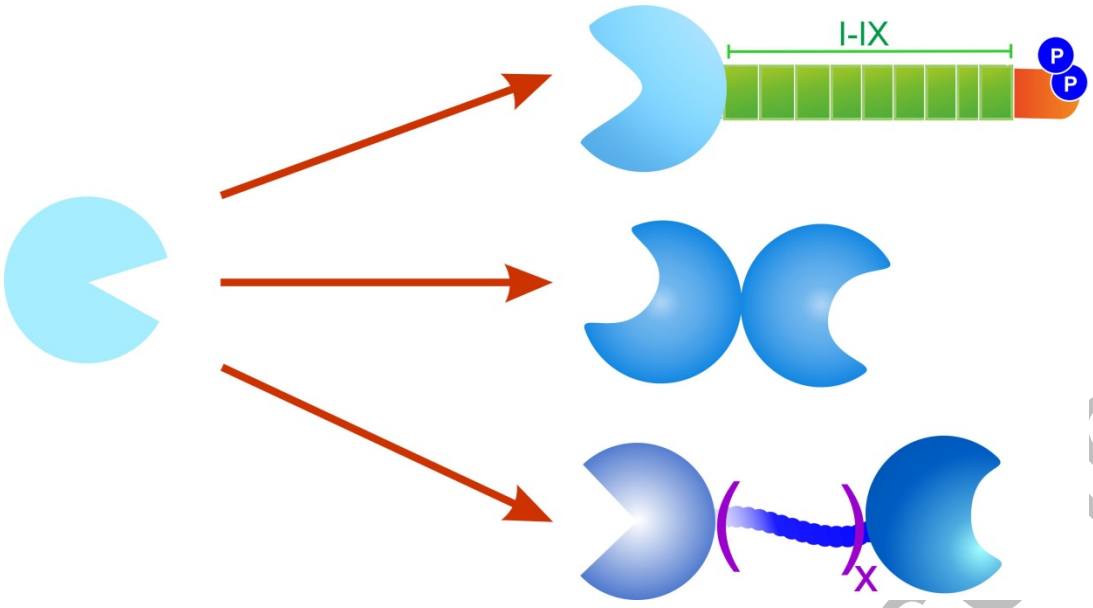


Figure1

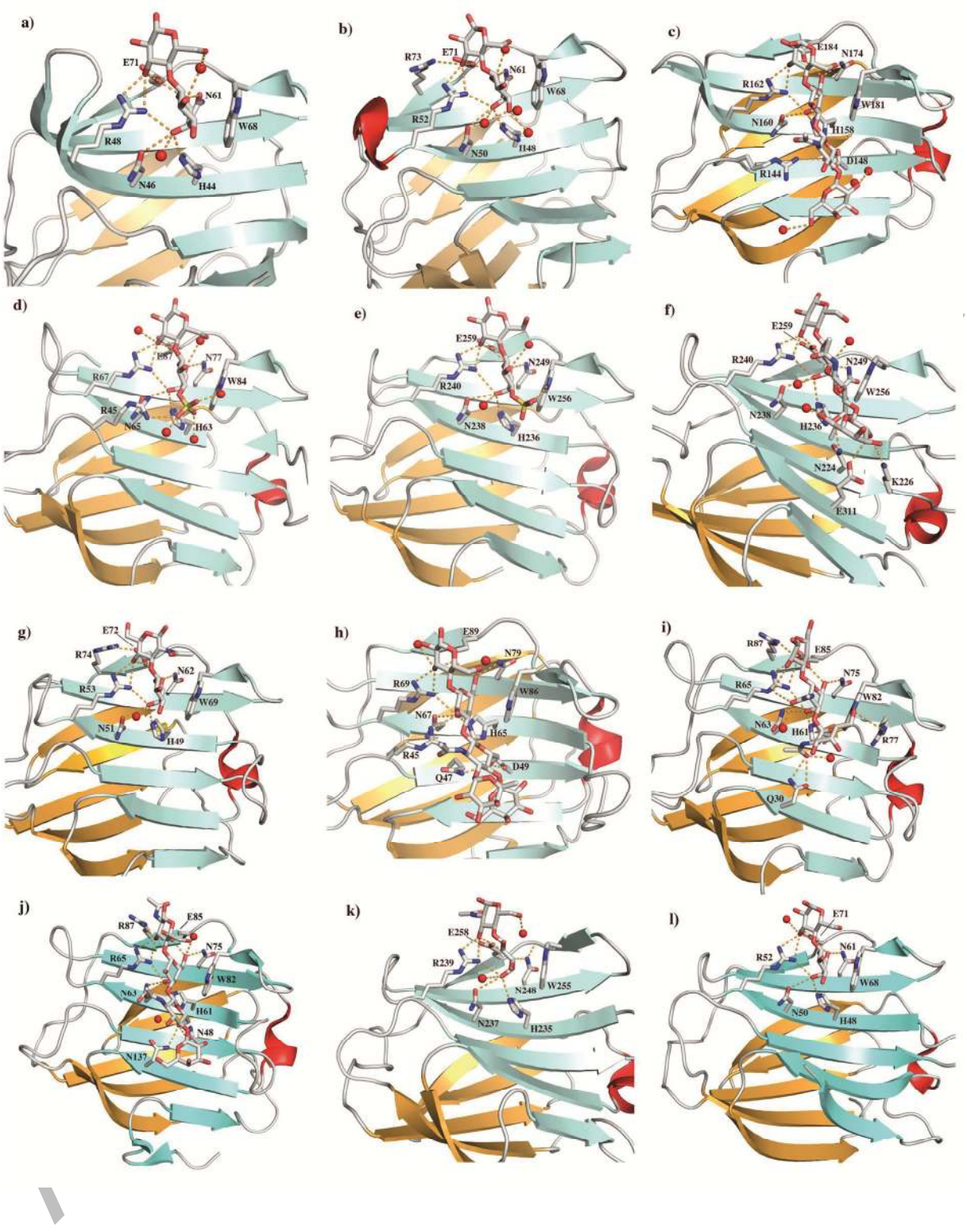


Figure2

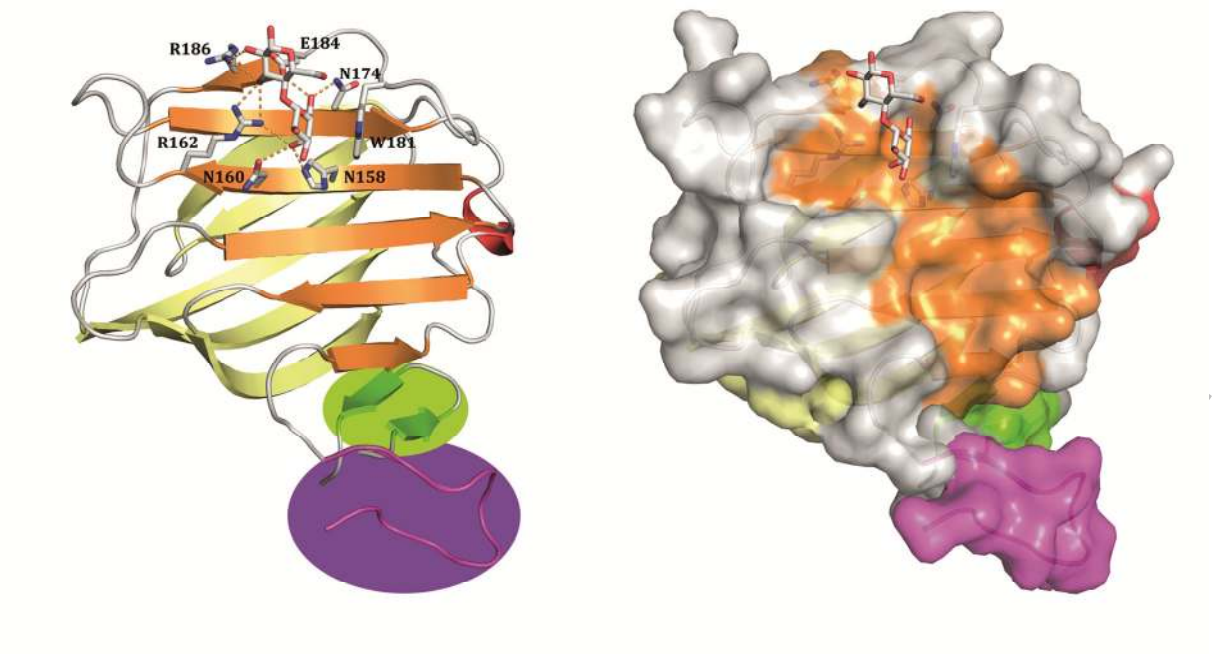


Figure3

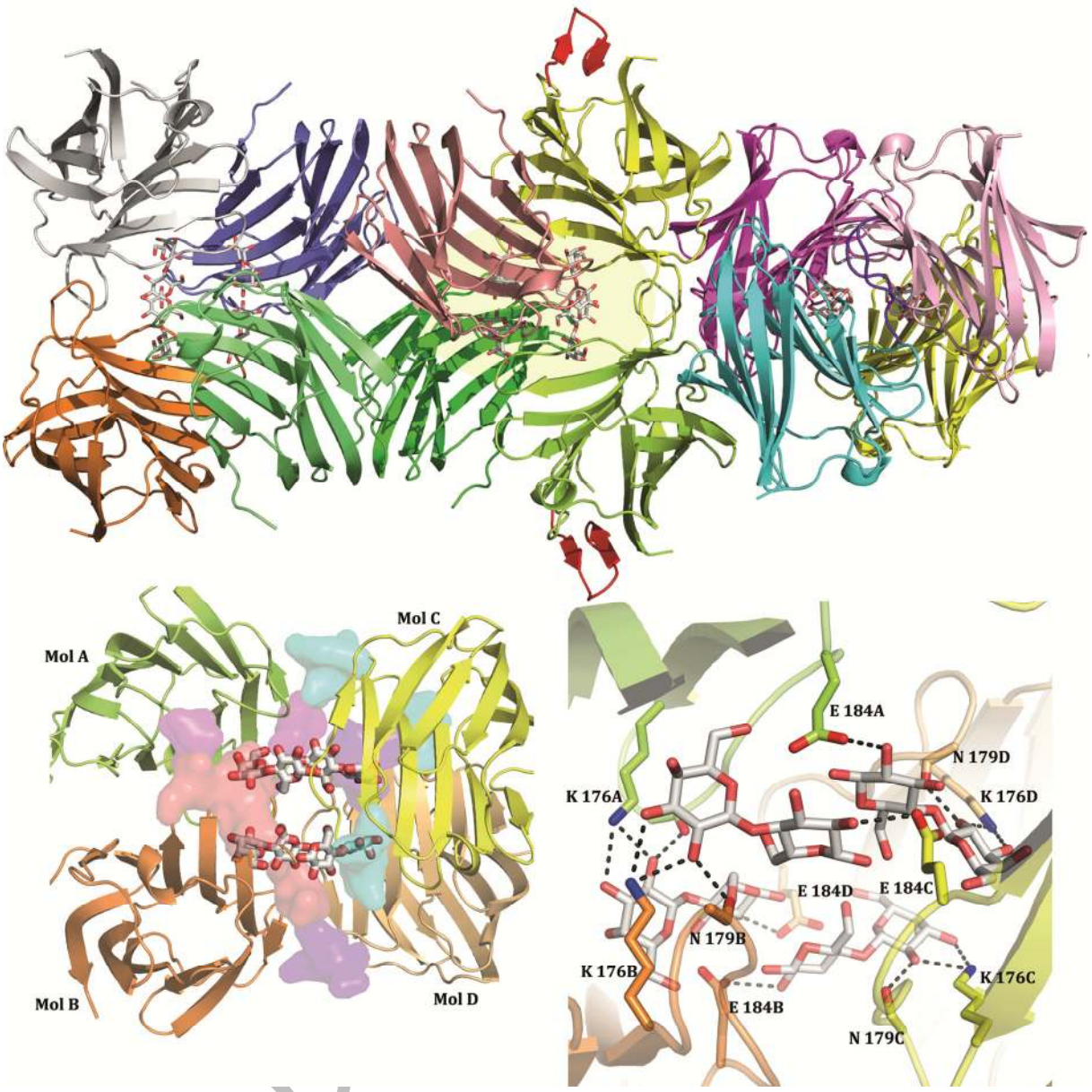
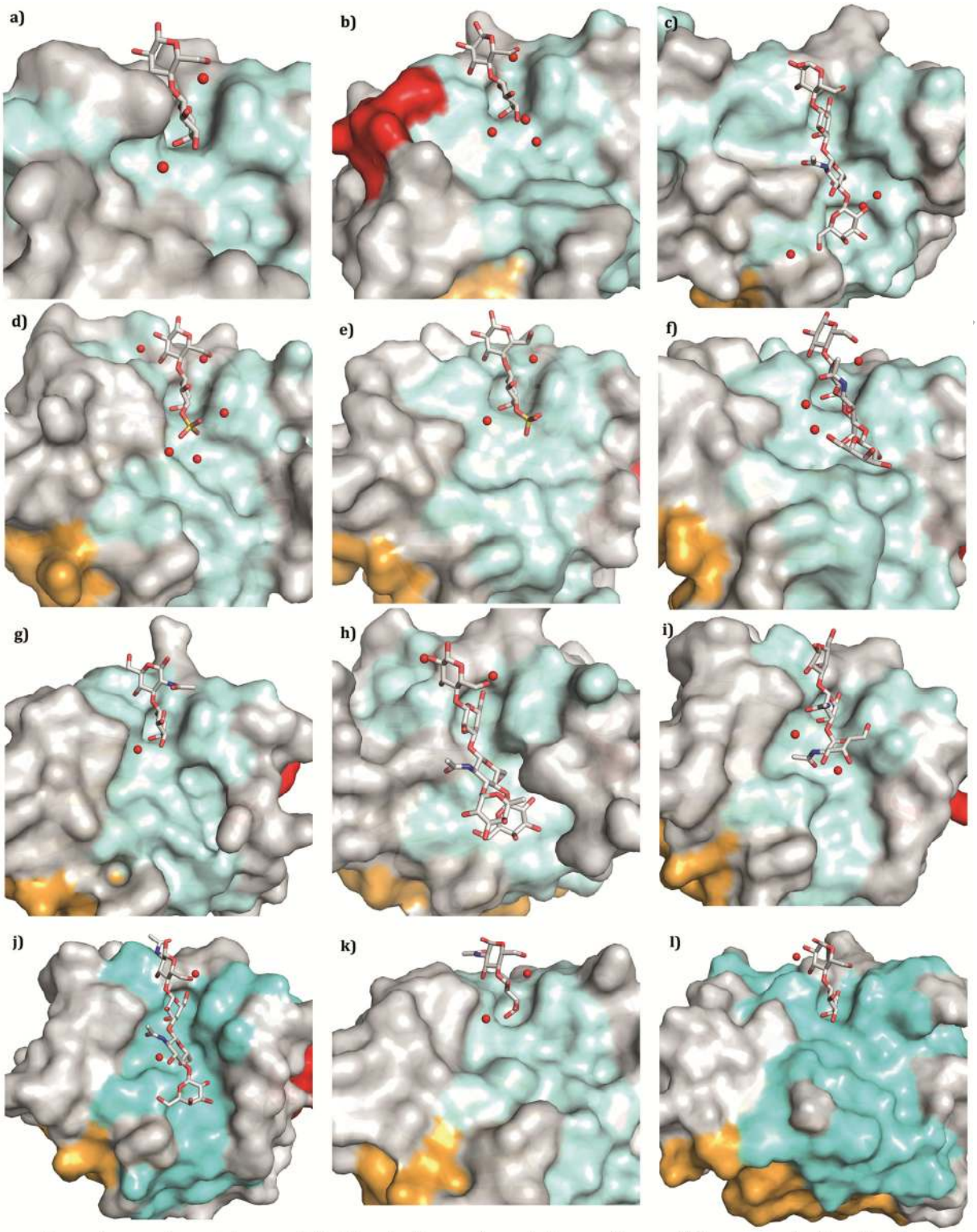


Figure4



Supplementary Figure S1. Illustration of protein surface of the panel of galectins given in Figure 1 (for details on the nature of galectin in each case, please see legend of that figure).