

Galectins: Structures, Binding Properties and Function in Cell Adhesion

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1. Introduction

Galectins are nearly ubiquitous distributed β -galactoside binding proteins which share a common amino acid sequence, the carbohydrate recognition domain (CRD) (Barondes et al., 1994a; Cooper, 2002; Elola et al., 2007; Hirabayashi & Kasai, 1993; Hughes, 2001; Klyosov et al., 2008; Leffler et al., 2004). They are evident in vertebrates, invertebrates and also protists, implying fundamental functions of these lectins (Hirabayashi & Kasai, 1993). Some galectins are distributed in a variety of different tissues, others are more specifically expressed (Cooper, 2002).

Galectins are known to perform high diversity of functions inside the cells and in the extracellular space. They are regulators of cell cycle, inflammation, immune responses, cancer progression, cell adhesion, cell signalling events and so on. The different functions are performed either by protein-protein or by protein-glycan interactions (Almkvist & Karlsson, 2004; Danguy et al., 2002; Elola et al., 2007; Hernandez & Baum, 2002; Hughes, 2001; Ilarregui et al., 2005; Liu et al., 2002; Liu & Rabinovich, 2005; Rabinovich et al., 2002b; Rabinovich & Toscano, 2009; Vasta, 2009).

Different excellent reviews focus on the wide-spread functions of galectins such as tumor progression, cell signalling or inflammation (Garner & Baum, 2008; Hernandez & Baum, 2002; Liu et al., 2002; Liu & Rabinovich, 2005; Nangia-Makker et al., 2008; Rabinovich et al., 2002a; Rabinovich & Toscano, 2009; van den Brule et al., 2004; Vasta, 2009). Review articles discussing functions of galectins in cell adhesion events and their role as matricellular proteins for the crosslinking of extracellular matrix components have also been published (Elola et al., 2007; Hughes, 2001). The function of galectins in the assembly of the extracellular matrix as well as in cell adhesion and cell signalling processes shows their potential as mediators for cell adhesion and proliferation on biomaterial surfaces. Galectins are interesting candidates for the functionalisation of biomaterial surfaces as they can promote the primary binding event of cells to foreign materials and influence specific signalling processes. In this article we want to analyse the potential use of galectins (explained by the examples of galectin-1, -3 and -8) in biomaterial research and application.

2. Families and structures of galectins

Galectins are defined by their β -galactoside binding ability and their common sequence of about 130 conserved amino acids. This sequence homology results in a similar overall three-

dimensional structure of the carbohydrate recognition domain (CRD) (Barondes et al., 1994a; Barondes et al., 1994b). Several human galectin CRDs have been characterised by crystallography, including those of human galectin-1, galectin-3 and the N-terminal domain of galectin-8 (Ideo et al., 2011; Kishishita et al., 2008; Lobsanov et al., 1993; Lopez-Lucendo et al., 2004; Seetharaman et al., 1998). The C-terminal domain of galectin-8 has been investigated by NMR (Tomizawa et al., 2008). All of them show a globular fold consisting of two anti-parallel β -sheets with five to six strands respectively (Ideo et al., 2011; Kishishita et al., 2008; Lobsanov et al., 1993; Lopez-Lucendo et al., 2004; Seetharaman et al., 1998; Tomizawa et al., 2008). The CRDs analysed so far consist of three consecutive exons, with most of the conserved amino acids encoded on the middle one (Cooper & Barondes, 1999; Houzelstein et al., 2004).

2.1 Galectin families

Regarding their overall structure galectins are clustered in three families: a) prototype galectins consisting of one CRD, b) chimera-type galectins with one CRD and a non-lectin domain whose only member known so far is galectin-3, and c) tandem-repeat galectins which have two different CRDs linked by a short peptide (see Fig. 1) (Hirabayashi & Kasai, 1993; Leffler et al., 2004).

In this review we want to focus on galectin-1, -3 and -8 as representatives of the three galectin families.

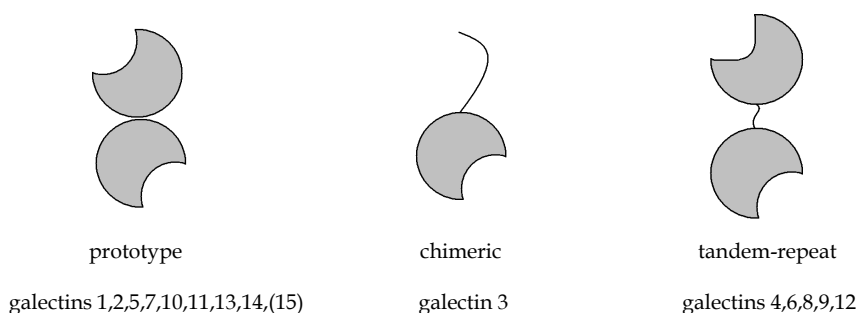


Fig. 1. Galectin families regarding their overall structure (modified from Barondes et al. 1994) (Al-Ansari et al., 2009).

Galectins are either divalent regarding their intrinsic protein structure (tandem-repeat galectins such as galectin-8) or form homotypic di- to oligomers through site-specific interactions (prototype and chimera galectins such as galectin-1 and -3). Different galectin-8 isoforms represent either prototypic or tandem-repeat type galectins depending on splice variants. Some galectin-8 splice variants consist only of the N-terminal CRD and different elongations without a second CRD. Those variants can rather be grouped to the prototypic galectins (Bidon et al., 2001; Al-Ansari et al., 2009; Zick et al., 2004). Prototype galectins such as galectin-1 form homodimers through hydrophobic interactions at the N-terminal amino acid residues (Cho & Cummings, 1997; Lobsanov et al., 1993). Dimerisation occurs as equilibrium reaction depending on protein concentration but independent of available soluble ligands (Cho & Cummings, 1995). In contrast the chimeric galectin-3 forms oligomers (most likely pentamers) via its N-terminal collagen-like extension after ligand-binding (Ahmad et al., 2004a; Birdsall et al., 2001; Nieminen et al., 2008).

2.2 Carbohydrate recognition domains

The carbohydrate recognition domain is highly conserved throughout different galectins and organisms.

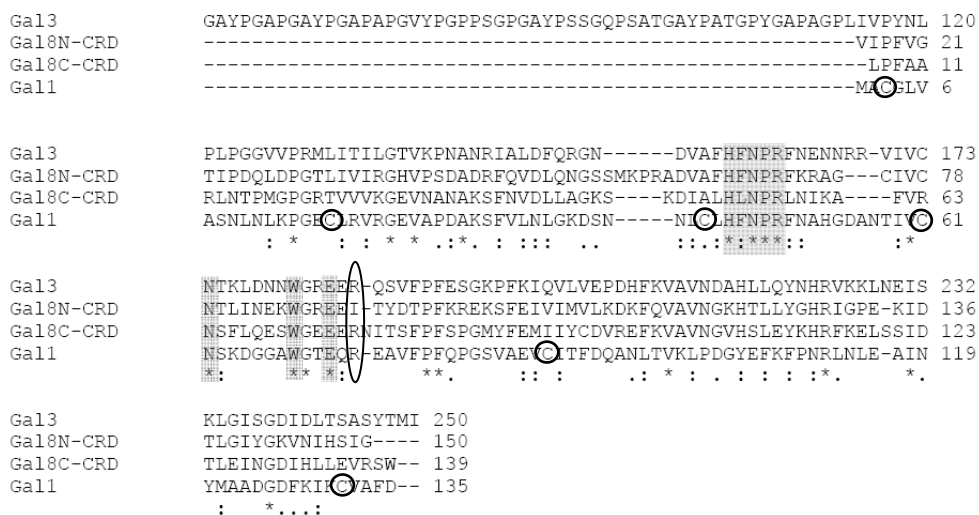


Fig. 2. Sequence alignment of human galectin-1, -3 and the single carbohydrate recognition domains of galectin-8 (residues 1-150 and 221-359 of isoform a). Used sequences are galectin-1 (NP_002296), galectin-3 (P17931) and galectin-8 isoform a (NP_963839) as published on <http://www.ncbi.nlm.nih.gov/protein>. Completely conserved amino acids are marked with an asterisk, conserved substitutions are marked with a colon and semi-conserved substitutions with a simple dot. Important amino acids mentioned in the following text are additionally highlighted: Conserved amino acids of the binding pocket are highlighted in grey; residues with importance for the binding are labelled in an ellipse; galectin-1 cysteine residues are marked with circles. The alignment has been performed using ClustalW2 at <http://www.ebi.ac.uk> using the default settings (Chenna et al., 2003; Larkin et al., 2007).

The conserved amino acids are directly involved in carbohydrate binding either by the formation of hydrogen bonds or van der Waals interactions with the sugar moiety. Most of the conserved amino acids form hydrogen bonds with the bound sugar unit. An important sequence motif in this context is His(158)-Asn(160)-Arg(162) (numbering according to human galectin-3, see Fig. 2). Those three amino acids have been found to form hydrogen bonds with the bound galactose residue for example in galectin-1 (Lobsanov et al., 1993), galectin-3 (Diehl et al., 2010; Seetharaman et al., 1998), and galectin-8 N-CRD (Ideo et al., 2011; Kishishita et al., 2008) (see Fig. 3). The sequence motif can also be found in galectin-8

C-CRD but as no x-ray crystallography is available for this CRD the hydrogen bridges have not been verified yet. Additional residues are involved in the conserved binding process either by hydrogen bonding (Glu184, Asn174, numbering according to human galectin-3, see Fig. 3) or van-der-Waals interaction (Trp181, numbering according to human galectin-3) (Di Lella et al., 2009; Diehl et al., 2010; Lobsanov et al., 1993; Seetharaman et al., 1998).

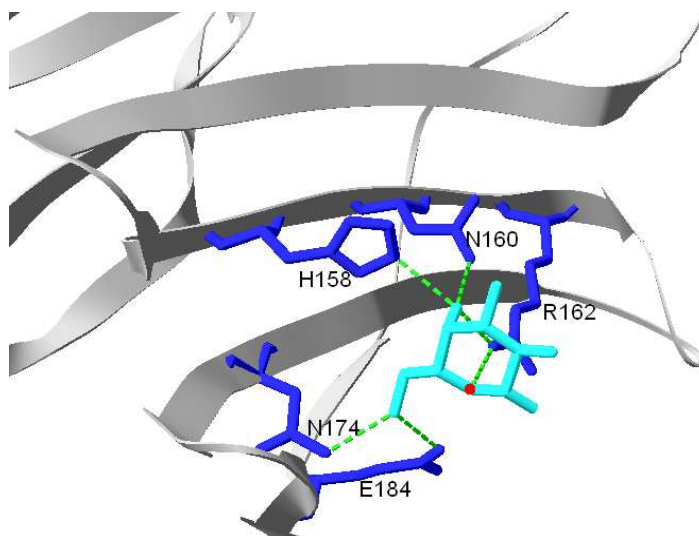


Fig. 3. Human galectin-3 with bound galactose unit of LacNAc **PDB 1KJL**. H-bondings are shown as dotted lines for residues H158 (C4-OH), N160 (C4-OH), R162 (C4-OH and intramolecular O-atom), N174 (C6-OH), E184 (C6-OH) (Seetharaman et al., 1998; Sörme et al., 2005). Picture made with SwissProt pdb viewer (Guex & Peitsch, 1997).

The importance of the mentioned H-bonding amino acids has been proven by site-directed mutagenesis performed with human galectin-1. In those experiments the change of single amino acids involved in H-bonding eliminates the binding to lactose-sepharose and/or asialofetuin (Hirabayashi & Kasai, 1991; Hirabayashi & Kasai, 1994). Although binding is not completely abolished, significant influence of the conserved Trp residue for sugar binding was also proven in bovine and human galectin-1 (Abbott & Feizi, 1991; Hirabayashi & Kasai, 1991).

Arg186 is not completely conserved throughout the different galectins (see Fig. 2, ellipse). The N-terminal domain of galectin-8 for example presents an Ile at the corresponding position resulting in a differing fine specificity for glycans. Due to this mutation galectin-8 N-CRD favours lactose structures over LacNAc type II structures in the binding site. Thereby different biological functions of galectin-8 in contrast to other galectins such as galectin-3 are regulated (Salomonsson et al., 2010). Specific binding preferences resulting from the differences in amino acid sequence will be discussed in chapter 4 in more detail.

2.3 Other specific features of galectins

2.3.1 Secretion

Different tissues are known to produce galectins and most of them secrete parts of the cytosolic galectin pool. The amount of secreted galectin depends on cell type, differentiation status and can be regulated by external triggers (Cooper, 2002; Hughes, 1999). Examples of galectin producing cells with relevance for regenerative medicine are beside others neurons, epithelial cells of several tissues and liver cells, which produce either several different galectins or a specific subset of galectins (Dumic et al., 2006; Hughes, 1999).

Galectins act intra- and extracellularly. As known so far they are secreted via a non-classical mechanism which is not fully understood yet. They lack classical signalling sequences for specific localisation but can be found in the outer cellular space as well as inside the cells even located in the nucleus (Hughes, 1999). Although the complex regulation of secretion remains still elusive some explanations have been found: Galectin-1 secretion depends on the binding to a counter-receptor molecule and does not involve plasma membrane blebbing (Seelenmeyer et al., 2005; Seelenmeyer et al., 2008). Galectin-3 secretion seems also to be regulated by binding to other proteins such as chaperons and subsequent vesicular secretion (Hughes, 1999; Mehul & Hughes, 1997). The N-terminal-domain of galectin-3 is important for subcellular translocation and secretion of the protein (Gong et al., 1999).

2.3.2 Galectin-1: Importance of reducing conditions

The lectin activity of galectin-1 depends on reduced cysteine residues. Oxidised galectin-1 has no lectin activity but functions in the regeneration of nerve axons (Horie et al., 2004). Galectin-1 has six cysteine residues which are accessible to the solvent (see Fig. 2). The removal of the most accessible cysteine (Cys2) (Lopez-Lucendo et al., 2004) - or better all cysteine residues - enhances protein stability under both reducing and non-reducing conditions significantly (Cho & Cummings, 1995; Nishi et al., 2008), while none of them is necessary for lactose binding as shown by site directed mutagenesis and x-ray crystallography (Hirabayashi & Kasai, 1991; Lopez-Lucendo et al., 2004).

2.3.3 Galectin-3: The only known chimera type galectin

Galectin-3 has some specific properties due to its unique structure. Galectin-3 consists of three parts: 1) a N-terminal 12 amino acid leader sequence containing two phosphorylation sites, 2) a proline and glycine rich collagen like domain necessary for oligomerisation and 3) the carbohydrate recognition domain (Ahmad et al., 2004a; Dumic et al., 2006; Kubler et al., 2008; Mehul & Hughes, 1997; Nieminen et al., 2008). The first few amino acids forming the leader peptide are important for the subcellular localisation and secretion of the protein (Gong et al., 1999). Moreover phosphorylation of Ser6 seems to regulate affinity for different ligands and thereby cellular activity of galectin-3 (Dumic et al., 2006; Mazurek et al., 2000; Szabo et al., 2009; Yoshii et al., 2002). Galectin-3 can be cleaved by different proteases such as metalloproteinases-2 and -9 (gelatinases A and B respectively), metalloproteinase-13 (collagenase-3) and with low activity metalloproteinase-1 (collagenase-1) separating the full-length CRD from the N-terminal extension (Guévremont et al., 2004; Ochieng et al., 1994). The main cleavage position is located between Ala62 and Tyr63 while other cleaving sites are only recognised by some specific proteases to lesser extend (Dumic et al., 2006;

Guévremont et al., 2004; Ochieng et al., 1994). The single CRD is mainly described to have an increased affinity for different carbohydrates such as *N*-acetyllactosamine, the glycoprotein asialofetuin or glycans presented on endothelial cells but to have less biological activity as it loses the ability to form oligomers. This reveals the possible regulatory function of galectin-3 cleavage (Dam et al., 2005; Dumić et al., 2006; Ochieng et al., 1998a; Shekhar et al., 2004). In terms of this regulation it is suggested that the single galectin-3-CRD binds with high affinity to glycans on cell surfaces thereby blocking these interaction partners for full-length galectin-3 binding. After this blockage the full-length protein cannot perform its physiological functions anymore. In this way galectin-3 cleavage could act as down-regulation of galectin-3 function (John et al., 2003; Shekhar et al., 2004).

2.3.4 Galectin-8: Several isoforms of a tandem-repeat galectin

The specific properties of galectin-8 are also implied in its structure and the different isoforms arising from it. At least 6 different isoforms are described so far of which some only consist of the N-terminal CRD with an extension and others consist of both CRDs linked by different hinge domains (Bidon et al., 2001; Delgado et al., 2011; Zick et al., 2004). The two galectin-8 CRDs show approximately 35% sequence similarity but reveal different fine specificity for glycan structures. Therefore galectin-8 can act as “hetero-bifunctional crosslinking agent” (Zick et al., 2004). The length and structure of the linker domain has direct influence on the biological function (Levy et al., 2006). Moreover the linker domain regulates susceptibility to protease cleavage. It was for example shown that a long linker can be cleaved by thrombin while shorter linker variants are not substrate for this protease (Nishi et al., 2006).

3. Glycan binding assays for galectins

As galectins play a fundamental role in cell adhesion, cell signalling, inflammation, tumor progression etc. there is an enormous interest in the evaluation of galectin-glycan interactions regulating those functions.

3.1 Comparison of different common assays

Various assay set-ups have been designed to analyse the binding behaviour of different galectins to specific glycan structures. Binding assays can be subdivided regarding the presentation of the different binding partners: 1) the glycan structure is immobilised, 2) the galectin is immobilised and 3) both binding partners are soluble.

The chosen assay format influences the data generated as each assay set-up has its own advantages and disadvantages (Rapoport et al., 2008):

Assays in which one of the binding partners is immobilised raise the problem that the amount of this ligand is not completely known. Moreover it is possible that side interactions with the surface occur or that the conformation and flexibility of the bound partner differ slightly from its soluble parameters. The natural oligomerisation of galectins is blocked after immobilisation. Beside this the presentation of the immobilised binding partner is multivalent which influences the binding (Sörme et al., 2004). This can be useful for the glycan structures, as they are multivalently presented in nature as well, but not for galectins. Examples for studies with immobilised glycans or glycoproteins are glycan arrays, ELISA

assays and surface plasmon resonance (Appukuttan, 2002; Blixt et al., 2004; Bohorov et al., 2006; Ideo et al., 2003; Munoz et al., 2010; Song et al., 2009b; Stowell et al., 2008a). For glycan arrays it can be important which linker is used to bind the glycan epitopes to the surface (length, chemical structure). Moreover it is possible to use chemically and/or enzymatically produced ligands as well as glycans from natural compounds like glycopeptides and glycolipids (Blixt et al., 2004; Bohorov et al., 2006). The latter allows the analysis of complex and even unknown glycan structures of different cells (Song et al., 2009a; Song et al., 2009b; Song et al., 2010). Immobilised galectins are for example used in frontal affinity approaches and ELISA assays (Hirabayashi et al., 2002; Sörme et al., 2002).

Variations of binding assays with immobilised partners are assays in which the surface binding is inhibited by a soluble ligand. Such inhibition studies of surface interactions allow a direct read-out of IC₅₀ values and thereby the direct comparison of relative affinities (Sörme et al., 2002). For the calculation of affinity constants assumptions have to be made to simplify calculations which might not be correct for each single interaction measurement. Additionally the problems mentioned before still persist (Sörme et al., 2004).

Most assays with one immobilised component as well as some direct interaction assays are based on the read-out of a fluorescence signal or other labels. Therefore either the galectin or the glycan structures have to be labelled. This leads to some additional problems: If the glycan is chemically labelled the linker or label itself can alter the binding affinity with specific effects for different galectins (Sörme et al., 2004). Therefore the affinity constants measured do not exactly fit to the unmodified glycan structures. Moreover the labelling of glycans is time-consuming. The labelling of galectins can also alter the binding specificities. It is in most cases done by random chemical modification of specific functional groups such as amino or thiol functionalities (Carlsson et al., 2007; Patnaik et al., 2006; Rapoport et al., 2008; Salomonsson et al., 2010; Song et al., 2009b; Stowell et al., 2008a; Stowell et al., 2008b). Although this labelling is assumed not to influence binding specificity or inactive galectins are removed after the labelling reaction, binding and oligomerisation still might be slightly affected. Moreover lot-specific aberrations between different labelling reactions occur. Labelled galectins are for example used for glycan arrays and ELISA-type set-ups (Blixt et al., 2004; Carlsson et al., 2007; Rapoport et al., 2008; Salomonsson et al., 2010; Song et al., 2009b; Stowell et al., 2008a) while fluorescence labelled glycans are used in frontal affinity chromatography or fluorescence polarisation (Carlsson et al., 2007; Hirabayashi et al., 2002; Salomonsson et al., 2010; Sörme et al., 2004).

Assays using both binding partners in its soluble form overcome most of the mentioned problems. But although those assays have different advantages the results cannot directly be compared with the natural set-up in which the glycans are immobilised on glycoproteins or glycolipids and thereby multivalently presented. Fluorescence polarisation is one of these methods measuring direct interactions of ligands in solution, but facing negative side effects of glycan labelling. Similarly, titration calorimetry also measures the interaction of two soluble binding partners. As for titration calorimetry no labelling reaction has to be performed this assay set-up might be considered as the one with fewest problems. But needed galectin concentrations for this test are usually (but not always) in high micromolar ranges and therefore above the physiological range. In this concentration range galectins tend to oligomerise, aggregate or precipitate (Ahmad et al., 2004b; Bachhawat-Sikder et al., 2001; Cho & Cummings, 1995; Dam et al., 2005; Sörme et al., 2004). Moreover titration

calorimetry experiments are suitable for comparative studies of different glycans but do not lead to accurate calculation of affinity constants (Ahmad et al., 2004b). Another way to determine the direct interaction of soluble galectins and glycans is the use of hemagglutination assays, but those are limited to multivalent glycans or the inhibition of interactions between galectins and multivalent glycans or erythrocytes (Ahmad et al., 2004a; Ahmad et al., 2004b; Appukuttan, 2002; Giguere et al., 2008).

3.2 Determined fine specificity of galectin-1, -3 and the two galectin-8 CRDs

Although the general fold of all galectins is highly conserved, single galectins are characterised by specific binding interactions with single carbohydrate ligands. Differences in fine specificity have been analysed using different binding assays (as mentioned above). Moreover extensive theoretical evaluation of the putative interactions between single amino acids and functional groups of the bound glycan has been done by modelling and calculation. Some specific ligands with high affinity for the single galectin CRDs are mentioned in Table 1.

The recognition of galactose is common for all galectins but the interaction with the monosaccharide alone is very weak (Carlsson et al., 2007; Knibbs et al., 1993; Salameh et al., 2010). Disaccharides containing galactose β -glycosidic bound to GlcNAc, Glc or GalNAc are bound with significantly increased affinities. Different galectins thereby show high affinity to specific disaccharides. Galectin-3, galectin-1 and the C-terminal CRD of galectin-8 bind preferentially LacNAc units of type I and type II while the N-terminal CRD of galectin-8 shows highest affinity for lactose (Carlsson et al., 2007; Ideo et al., 2011; Salomonsson et al., 2010).

Extensions of the bound galactose moiety effect glycan binding in dependence on the galectin. Galectin-3 tolerates due to its enlarged binding pocket extensions at the galactose 3'-OH-group for example repetitive LacNAc (type II) -structures (poly-LacNAc), showing even higher affinities for repetitive LacNAc structures compared to single LacNAc units (Hirabayashi et al., 2002; Rapoport et al., 2008; Salomonsson et al., 2010). In contrast galectin-1 recognises single LacNAc units presented at the non-reducing terminus of glycans not showing preference for extended poly-LacNAc glycans (Leppänen et al., 2005). Most authors agree that galectin-1 is not able to bind internal galactose moieties in poly-LacNAc-glycans (GlcNAc- β 3-Gal- β 4-GlcNAc) (Leppänen et al., 2005; Stowell et al., 2004; Stowell et al., 2008a) but depending on the assay set-up some publications report affinity to this sugar unit (Di Virgilio et al., 1999; Zhou & Cummings, 1993). These different results prove the importance of evaluation of the test set-up and critical examination of the measured binding data.

Other extensions at the 3'-OH-group of galactose such as sulphate or neuraminic acid increase the affinity of galectin-3, galectin-1 and especially galectin-8 N-CRD to the core disaccharide (Carlsson et al., 2007; Sörme et al., 2002; Stowell et al., 2008a). In contrast the C-terminal galectin-8 domain fails to bind 3'-sulfated or 3'-sialylated galactose (Ideo et al., 2003).

Modification at the 6'-OH-group for example with neuraminic acid reduces binding of all four discussed galectin CRDs (Ideo et al., 2003; Stowell et al., 2008a). Therefore α 6-sialylation is discussed as regulatory modification for galectin-mediated functions (Zhuo & Bellis, 2011). Galectin-3 and galectin-8 C-CRD show high affinity for blood-group antigens (Hirabayashi et al., 2002; Yamamoto et al., 2008)

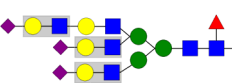
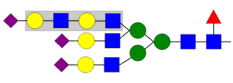
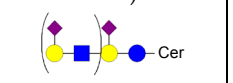

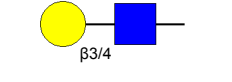
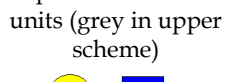
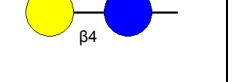
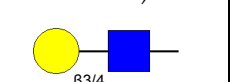
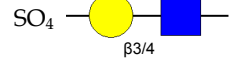
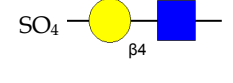
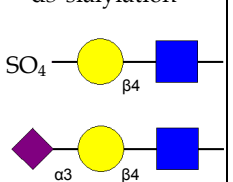
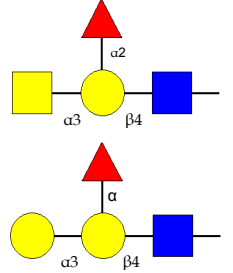
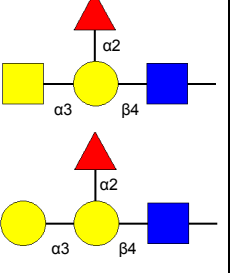
Galectin-1	Galectin-3	Galectin-8 N-CRD	Galectin-8 C-CRD
<p>Complex <i>N</i>-glycans Increasing affinity with increasing number of antennas</p> 	<p><i>N</i>-glycans, preferred poly- LacNAc</p> 	<p><i>N</i>-glycans and glycosphingolipids (e.g. GM3 and GD1a)</p> 	<p><i>N</i>-glycans, preferred poly- LacNAc</p> 
<p>Non-reducing terminal LacNAc type I or II (grey in upper scheme)</p> 	<p>Non-reducing terminal and internal LacNAc, high affinity for repetitive LacNAc- units (grey in upper scheme)</p> 	<p>Preference for lactose (but also binding to LacNAc- units)</p> 	<p>LacNAc type I or II high affinity for repetitive LacNAc- units (grey in upper scheme)</p> 
<p>3-O-sulfation</p> 	<p>3 O-sulfation</p> 	<p>3 O-sulfation and alpha3-sialylation</p> 	
	<p>Blood group A and B antigens</p> 		<p>Blood group A and B antigens</p> 

Table 1. Preferred ligands of the single carbohydrate recognition domains of galectin-1, -3 and -8 following Rapoport et al. 2002. Symbols according to the consortium of functional glycomics (Brewer, 2004; Carlsson et al., 2007; Dell, 2002; Hirabayashi et al., 2002; Ideo et al., 2003; Ideo et al., 2011; Leppänen et al., 2005; Patnaik et al., 2006; Rabinovich & Toscano, 2009; Rapoport et al., 2008; Salomonsson et al., 2010; Stowell et al., 2004; Stowell et al., 2008a; Yamamoto et al., 2008)

4. Glycoproteins as binding partners of galectins

Galectins can act intracellular or in the extracellular space, where they have different functions regulated by protein-protein or protein-glycan interactions. In the extracellular space they interact with different glycoproteins influencing cell adhesion, signalling and proliferation events. Thereby they interact with ECM-glycoproteins forming the extracellular matrix and with glycosylated transmembrane or membrane associated proteins on the cell surface (table 2). Following we present some selected binding partners of the three different galectins discussed so far.

4.1 ECM glycoproteins as binding partners of galectins

Different extracellular matrix proteins contribute to structural and functional aspects of the extracellular space. Galectin-1 interacts strongly with different extracellular matrix proteins. It has affinity to several glycoproteins as with increasing affinity osteopontin, vitronectin, thrombospondin, cellular fibronectin and laminin (Moiseeva et al., 2003). Most of these interactions depend on the carbohydrate recognition domain and can be inhibited with soluble glycan ligands (Cooper, 1997; Moiseeva et al., 2003; Ozeki et al., 1995; Zhou & Cummings, 1993). Galectin-3 also shows high affinity for some ECM-glycoproteins (Dumic et al., 2006; Kuwabara & Liu, 1996; Massa et al., 1993; Matarrese et al., 2000; Ochieng et al., 1998b; Sato & Hughes, 1992). The best binding candidates fibronectin and laminin are heavily glycosylated (5-7% and at least 12-15% respectively), carrying mainly *N*-glycans (Paul & Hynes, 1984; Tanzer et al., 1993). *N*-glycans are among the main binding partners of galectin-1, -3 and -8 (Patnaik et al., 2006) (although galectin-8 also shows high affinity to some glycosphingolipids (Ideo et al., 2003; Yamamoto et al., 2008)). One third of laminin *N*-glycans is composed of repetitive “*N*-acetylglucosamine” units (shown for mouse EHS-laminin) which are preferentially recognised by galectin-3 but also by galectin-1 and to less extent galectin-8 (Arumugham et al., 1986; Hirabayashi et al., 2002; Knibbs et al., 1989; Sato & Hughes, 1992; Zhou & Cummings, 1993). The other ECM-glycoproteins carry also *N*-glycans but are less glycosylated (Bunkenborg et al., 2004; Chen et al., 2009; Liu et al., 2005). For example osteopontin from human bone shows only two *N*-glycans with binding sites which are partially blocked by α 6-bound sialic acid (Ideo et al., 2003; Masuda et al., 2000; Stowell et al., 2008a). Another extracellular matrix protein interacting with galectin-1 and -3 is the Mac-2 binding protein or 90K antigen which influences adhesion processes (Sasaki et al., 1998; Tinari et al., 2001).

The different ECM-proteins which are bound by galectins can interact with other ECM-glycoproteins and/or integrins (Adams, 2001; Janik et al., 2010; Kariya et al., 2008; Singh et al., 2010). These interactions can lead to regulatory effects, lattice formation and signalling cascades.

4.2 Cell-surface glycoproteins as binding partners of galectins

Beside these soluble ECM components also some membrane-bound proteins are recognised by galectins. One of these is the lysosome associated membrane glycoprotein 1 (LAMP-1) which is known to carry several *N*-glycans partly presenting poly-lactosamine glycans recognised by galectin-3 and -1 (Chen et al., 2009; Do et al., 1990; Dong & Hughes, 1997). LAMP-1 is also known as CD107a. Several other membrane proteins associated in the cluster of differentiation such as CD3, 4, 7, 8, 43 and 45 which are presented on T-cells are

also recognised by galectin-1, showing the function of galectin-1 in immune response and inflammation (Liu, 2005; Nishi et al., 2008; Pace et al., 1999; Rabinovich et al., 2002a; Rabinovich et al., 2002b).

Binding partner	Gal	Cell type	Process	Reference
Integrin $\alpha 7\beta 1$	1	Skeletal muscle cells; Myoblasts	Influences integrin-laminin interaction	(Gu et al., 1994)
Integrin $\alpha 1\beta 1$	1	Vascular smooth muscle cells	Influences adhesion and migration	(Moiseeva et al., 1999)
Integrin $\alpha 3\beta 1$	8	e.g. endothelial cells	Influences cell adhesion and survival by modulating integrin-ECM interaction	(Hadari et al., 2000)
Cell recognition molecule L1; Myelin associated glycoprotein (MAG) Neural cell adhesion molecule (NCAM)	3	Neural tissue	Likely influences cell adhesion and signalling processes	(Probstmeier et al., 1995)
NG2 proteoglycan	3	Microvascular pericytes	Influences endothelial cell motility and morphogenesis	(Wen et al., 2006)

Table 2. Examples of cell-surface-glycoproteins interacting with galectins

This does not constitute a comprehensive list of cell-bound galectin-binding-glycoproteins, but just intends to show some examples which might be interesting for tissue engineering. Immune and tumor cells are not included in the list.

Similarly galectin-3 binds to CD98 on macrophages, CD66 on neutrophils and the T-cell receptor also showing functions in immune response and inflammation (Demetriou et al., 2001; Dong & Hughes, 1997; Dumic et al., 2006; Hughes, 2001). Other cell surface markers involved in cell-adhesion processes such as CD44 are bound by galectin-8 in a glycan-dependent manner underlining the importance of galectin-8 as matricellular protein involved in the regulation of cell-adhesion (Sebban et al., 2007).

All three galectins mentioned in this review are able to bind different integrin subunits. All bind to $\beta 1$ -integrins (Dumic et al., 2006; Furtak et al., 2001; Hughes, 2001; Sakaguchi et al., 2010; Zick et al., 2004). In this context galectin-3 binding to $\beta 1$ -integrins leads to an internalisation signal, regulating receptor amount on the cell surface and thereby influencing cell signalling aspects (Furtak et al., 2001). Other integrins such as $\alpha v\beta 3$ integrin on endothelial cells or the αM subunit on macrophages are also bound by galectin-3 (Dong & Hughes, 1997; Markowska et al., 2010). Galectin-8 is known to have a major function in integrin-binding and integrin-mediated signalling (Zick et al., 2004). The binding of galectin-8 N-CRD to the $\beta 1$ -integrin-subunit is especially good as high affinity $\alpha 2$ -3-

sialylated ligands are presented on this subunit (Diskin et al., 2009). Beside the β 1-subunit galectin-8 N-CRD also binds α 5 and some other integrin-subunits, but literature does not give a clear picture about the exact integrin binding partners. For example *N*-glycans on the α 4-subunit are once mentioned as main binding partner while other authors do not report binding to this subunit. Similar discrepancies were noticed for other subunits (Cárcamo et al., 2006; Diskin et al., 2009; Hadari et al., 2000; Nishi et al., 2003; Yamamoto et al., 2008). This might be explained by tissue- or cell-specific glycosylation patterns of the single subunits. In contrast to most interactions which are performed by the N-terminal galectin-8 CRD binding to the α M-subunit is performed by the C-terminal CRD (Nishi et al., 2003).

5. Galectin function in cell adhesion and cell migration

5.1 Principle function

Galectins can act pro- or antiadhesive for different cell types. They can either facilitate or reduce adhesion to other cells depending on different factors. Cell adhesion is enhanced if galectins crosslink glycosylated structures on one cell with glycans on other cells or the extracellular matrix. In contrast the adhesion is reduced if soluble galectins block available receptors for other binding interactions. This depends on one hand on galectin concentration. At high concentrations galectins may block all available receptors without interaction with each other which is necessary for crosslinking and therefore for adhesion (Elola et al., 2007). It is for example discussed that galectin-3 outbursts can lead to detachment of cells from the extracellular matrix as galectin-3 blocks integrin binding to ECM glycoproteins (Ochieng et al., 1998b). On the other hand it is important which receptors are available on the specific cell type used in the experiment and if those receptors interact more easily with the soluble galectins or with receptors on the surface the cell attaches to (Elola et al., 2007). Additional the oligomerisation state of the galectins plays an important role as they can either block receptors or crosslink molecules depending on their valency (Hughes, 2001). The oligomerisation is in case of galectin-1 depending on galectin concentration while galectin-3 stays monomeric in solution without ligand binding and builds pentamers after the binding reaction (Ahmad et al., 2004a; Cho & Cummings, 1995; Cho & Cummings, 1997; Morris et al., 2004; Nieminen et al., 2008). Moreover effects of single galectins can hardly be determined as most cell types co-express different galectins which might at least partially result in overlapping or opposite effects (Cooper & Barondes, 1999; Liu & Rabinovich, 2005).

In addition to direct binding of galectins to glycan structures on either membrane-bound receptors or ECM-glycoproteins, regulation of integrin amount, availability and affinity by galectin binding also contributes to adhesion events. Galectin-3 for example is able to increase amount and/or affinity of β 2-integrins on the cell surface on neutrophils, thereby regulating the binding to ECM glycoproteins recognised by integrins (Hughes, 2001; Kuwabara & Liu, 1996). Overexpression of galectin-3 correlates with enhanced surface expression of α 4 β 7 integrins resulting in increased cell adhesion (Matarrese et al., 2000). In contrast binding of galectin-3 leads to internalisation of β 1-integrins in breast carcinoma cells (Furtak et al., 2001). Moreover the clustering and residence time of other receptors on the cell surface is regulated by the formation of glycan-galectin lattices thereby regulating different signalling processes (Garner & Baum, 2008; Lau & Dennis, 2008; Rabinovich et al., 2007).

5.2 Selected examples of cell adhesion and motility regulated by galectins-1, -3 and -8

We here present only few examples of galectin function in cell adhesion and motility processes. The list is by far not complete. Other review articles focus more detailed on cell adhesion events mediated by galectins (Elola et al., 2007; Hughes, 2001).

Galectin-1 is an important factor for the adhesion and proliferation of neural stem cells and neural progenitor cells. The adhesion is mediated by the carbohydrate recognition domain and interaction of this binding domain with integrin $\beta 1$ subunit (Sakaguchi et al., 2006; Sakaguchi et al., 2010). Moreover galectin-1 can reduce the motility of immune cells which might explain parts of its anti-inflammatory effects (Elola et al., 2007; Liu, 2005; Rabinovich et al., 2002a; Rabinovich et al., 2002b).

One important function of galectin-3 is associated with angiogenesis (Nangia-Makker et al., 2000a; Nangia-Makker et al., 2000b). Galectin-3 increases for example angiogenesis by forming integrin $\alpha \beta 3$ lattices on the cell-surface leading to FAK regulated downstream signalling. Galectin-3 mediated angiogenesis depends on the growth factors VEGF and bFGF (Markowska et al., 2010). Another interesting function of galectin-3 is the chemotattraction of monocytes via a G-protein coupled receptor pathway and the role in eosinophil rolling to sites of inflammation (Rao et al., 2007; Sano et al., 2000). Most of those functions can only be performed by full length galectin-3 showing the importance of glycan binding and oligomerisation of the protein (Markowska et al., 2010; Sano et al., 2000). Different other biological activities are also depending on both N- and C-terminal domain (Nieminen et al., 2005; Ochieng et al., 1998a; Sano et al., 2000; Sato et al., 2002; Yamaoka et al., 1995). This proves the possibility of regulating galectin-3 function by protease cleavage as mentioned in chapter 2.3.3.

Galectin-8 has been assigned to matricellular proteins which are able to promote cell adhesion. CHO-cells on galectin-8 show similar binding kinetics as on fibronectin but differ in their formation of cytoskeleton (Boura-Halfon et al., 2003). Moreover the binding to galectin-8 triggers specific signalling cascades as Ras, MAPK and Erk pathway (Levy et al., 2003). A physiological function in human might be the modulation of neutrophil function. Galectin-8 promotes neutrophil adhesion by binding αM integrin and promatrix metalloproteinase-9. Moreover superoxide production which is essential for neutrophil function is triggered by galectin-8 C-terminal CRD (Nishi et al., 2003). Another galectin-8 function might be the promotion of angiogenesis as it was also shown for galectin-3. Galectin-8 increases tube formation *in vitro* and angiogenesis *in vivo* in dependence of its specific carbohydrate affinity at physiological concentrations. This regulatory function is at least partially depending on CD 166 (Delgado et al., 2011).

6. Galectins in biomaterial research

As discussed in chapter 5.1 galectins can act pro- and antiadhesive which *in vivo* seems to be mainly regulated by concentration and oligomerisation status of the galectins. In the context of biomaterial research it is also of huge importance if the galectins are immobilised or soluble presented. Immobilised galectins act mainly proadhesive as they crosslink the surface they are immobilised on with glycosylated structures on the cell-membrane. Soluble galectins can either facilitate or reduce adhesion for example to functionalised surfaces as discussed for the *in vivo* situation in chapter 5.1 depending on concentration, oligomerisation and cell type (respectively receptor availability on this cell type) (Elola et al., 2007).

The pro-adhesive properties of galectins have been shown several times. But only few efforts have been done to elucidate the potential of galectins as coatings for biomaterial surfaces. In contrast other components of the extracellular matrix are often used. Coatings with peptides from ECM proteins such as RGD or YIGSR peptide are one of the most common methods to modify biomaterial surfaces. Also coatings with complete ECM proteins or specific adhesion proteins have been investigated. Another important molecule class used in biomaterial research today are growth factors (Chan & Mooney, 2008; Shekaran & Garcia, 2011; Straley et al., 2010). The functionalisation with glycans or lectins seems to be underrepresented although their function in natural processes is well known. Only few studies show the potential of galectins and glycans as biomaterial coatings: The positive influence of galectins was shown for example as the coating of PLGA scaffolds with recombinant galectin-1 promotes adhesion and growth of immortal rat chondrocytes. Therefore this surface is mentioned to have potential as biomaterial in tissue engineering (Chen et al., 2005). The potential of glycans in biomaterial coatings has also been shown. For example galactose derivatives immobilised on material surfaces were proven to influence the growth and function of liver cells positively. But in this study the receptor molecules and mechanisms of signal transduction were not investigated and binding of an asialoglycoprotein receptor (and not galectin mediated binding) is assumed (De Bartolo et al., 2006). Another study shows combined use of immobilised glycans with galectins as it evidences positive effects of endogenous galectin-1 for adhesion of chondrocytes to a lactose-modified surface (Marcon et al., 2005). These findings prove the possible use of glycan and/or galectin modified materials for improved cell adhesion.

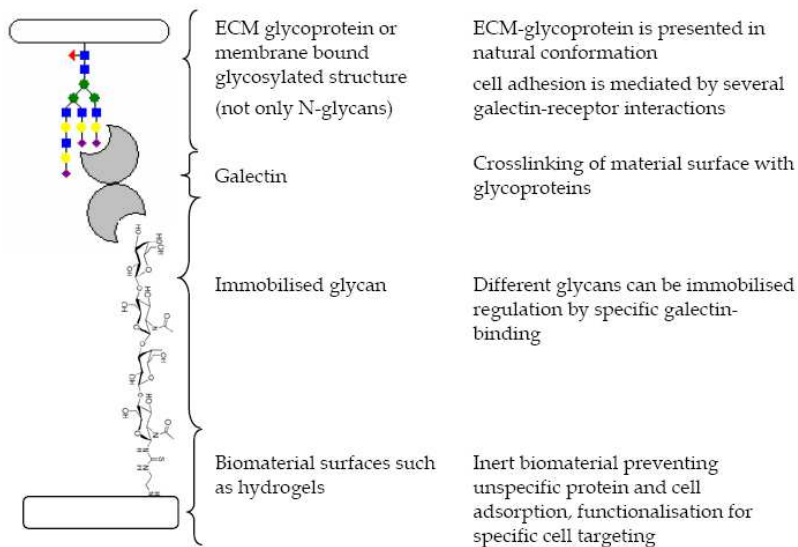


Fig. 4. Schematic representation of a possible biomaterial set-up using immobilised glycans as scaffold for subsequent galectin-mediated protein and cell-adhesion

Our recent work shows the potential of galectins and glycans in the preparation of biomaterial surfaces (figure 4). The assembly of an artificial extracellular matrix consisting of immobilised glycans, galectins and other extracellular matrix components was proven with a fungal model lectin (CGL2) (Sauerzapfe et al., 2009). In this approach poly-*N*-acetyllactosamine structures which are well known ligands for galectins (see chapter 3.2) are enzymatically produced. Those structures can be immobilised to different functionalised materials by a free amino group coupled to the reducing sugar. Concentration dependent binding of lectins to immobilised glycans was proven showing differences for specific glycan ligands. Lectin-mediated crosslinking of the surface with ECM-glycoproteins was also shown (Sauerzapfe et al., 2009). This galectin-mediated binding of ECM-glycoproteins leads to a natural presentation of these structures for subsequent adhesion of cells.

Our ongoing work focuses on the transfer of this set-up to applicable biomaterial surfaces. On the one hand recombinant human galectins are used instead of the fungal lectin to provide a more natural set-up (unpublished data). On the other hand the assembly of this artificial extracellular matrix is transferred to a special hydrogel surface. Star shaped NCO-sP(EO-stat-PO) is used as inert biomaterial which prevents unspecific protein adsorption and can be further functionalised with specific structures such as sugar molecules (Bruellhoff et al., 2010; Grafarend et al., 2011). On the basis of these glycans an artificial extracellular matrix composed of galectins and ECM-glycoproteins can be built up. Fibroblasts show excellent adhesion and cell spreading on these surfaces (Rech, Beer, Elling, Groll, manuscript in preparation).

7. Conclusion

The importance of galectins in cell adhesion and signal transduction has been shown in several investigations. Therefore a possible application of galectins in the assembly of a biomaterial surface mimicking the natural microenvironment seems to be obvious. Anyhow only few articles regarding the use of galectins in biomaterial research have been published. This might be explained by the fact that the fine regulation of galectin mediated cell adhesion and signalling is still not fully understood yet. Therefore it is important to evaluate galectin function under specified conditions to reduce or exclude the risk of unwanted inflammatory or carcinogenic effects.

Taking the presented literature and our own work regarding the biofunctionalisation of surfaces with glycans and galectins together, there is clear evidence that galectins play an important role in cell adhesion and proliferation on specifically functionalised biomaterial surfaces. However, further research has to be done to adopt the fundamental understanding of galectin-glycan mediated cell adhesion processes to an applicable biomaterial surface.

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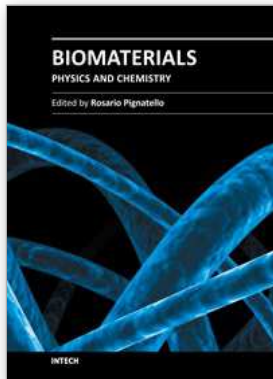
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