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Extracellular functions of galectin-3

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Galectin-3 has been suspected of modulating cell to extracellular matrix interactions in a novel fashion ever since it was first described. However, the rapid accumulation of research data in just the last 8 years alone has completely changed our perspective of this multifunctional protein. Its chimeric nature (consists of carbohydrate recognition and collagen like domains) somehow makes it suited to interact with a plethora of interesting extracellular matrix proteins some of which might enable it to cross the plasma membrane despite its lack of appropriate signal peptides. It is now becoming established as a mediator of signal transduction events on the cell surface as well as a mediator of a variety of extra-cellular processes such as kidney development, angiogenesis, neuronal functions, tumor metastasis, autoimmune disorders, endocytosis and possibly exocytosis. Nevertheless, it still retains its unique position as a mediator/modulator of cell to extracellular matrix adhesive interactions. Cells, particularly epithelial cells which lack galectin-3 expression, interact poorly with their extracellular matrices. In some of these processes, it functions as a matricellular protein, displaying both pro- and anti-adhesive properties.
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Abbreviations: **CRD, carbohydrate-recognition domain, M2BP, Mac-2 binding protein, ECM, extracellular matrix.**

Introduction

Galectin-3 is probably the most studied among the members of the galectin family. It is mainly a cytosolic molecule but can easily traverse the intracellular and the plasma membranes to visit the nucleus, mitochondria and be externalized despite its lack of classical localization signals at the amino terminal end of the molecule [1–3]. It has been associated with the splicing apparatus in the nucleus and so is thought to be involved in this process and perhaps the proliferative potential of the cell [4]. In both the cytosol and mitochondria, it interacts with anti-apoptotic signaling molecules such as Bcl-2 [5–7]. In the extracellular compartment, it has long been suspected of regulating cellular adhesion in a novel fashion. It has also been implicated in organogenesis, immune system and tumorigenesis [8–10]. In the mid 90's, scientific interest in this molecule was somewhat muted following the report from Dr. Poirer's laboratory, where they showed that galectin-3 knockout mice as well as galectin-1/galectin-3 double mutants did not manifest any obvious defects in implantation or early development. This study suggested that galectin-3 and its counter part galectin-1 are not obligatory housekeeping proteins and hence targeting them would not yield interesting physiological mechanisms [11].

Whereas galectin-3 null mice are still hot research items particularly in studies geared towards tumorigenesis and other pathological conditions, majority of normal adult tissues of mice with the exception of chondrocytes [12] can do without galectin-3. So, at what point in normal physiology is galectin-3 needed in the body? Is it more important and relevant only in pathological conditions such as during infection with microorganisms, diabetes, myocardial infarction, or tumor metastasis? Is it more relevant during implantation and fetal development in higher primates as compared to rodents? These are some of the research questions that need to be addressed. In the meantime, galectin-3 research is a fertile field and a number of loose ends still need to be tied before we can begin to look at the big picture. In this review, we will examine critically the data that has been reported regarding the extracellular functions of galectin-3. Its role in cellular adhesion will be discussed at length because of some lingering questions that are not yet clear.

Binding affinities of galectin-3 to various sugar residues

The biological activities of galectin-3 in the extracellular compartment mainly involve its interactions with various beta-galactoside containing glycans via its carbohydrate recognition

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Table 1. Affinity data for galectin-3 binding to glycoligands*

No.	*Ligand*	*Relative affinity*	
1	Lac	1.0**	1.0***
2	LacNAc	6.6	
3	2′-aFuc-Lac	3.0	
4	3-aGal-LacNAc	23.3	
5	3,6 $LacNAc_2$-Lac	29.1	
6	3-aGalNAc-(2-aFuc)-Gal	16.7	
7	3′-aGalNAc-(2′-aFuc)-Lac	17.3	
8	3′-NAc-LacNAc		11.6
9	3′-NBzl-LacNAc		97.6
10	3′-NSuc-LacNAc		105.0
11	3′-N(4-MeO-F_4-Bzl)-LacNAc		311.0

*Determined at 25–26°C.
**Direct interaction by microcalorimetry [14].
***Inhibition of oligosaccharide **#4**-HRP conjugate binding to Galectin-3 in ELISA [15].

domain (CRD). The CRD which encompasses 135 amino acid residues, is composed of 5 stranded (F1–F5) and 6 stranded (S1-S6a/6b) β-sheets, which associate in β-sandwich arrangement [13]. One of the long-term goals in galectin research is to identify effective inhibitors of their biological functions in the extracellular compartment. To this end, a number of synthetic schemes including a combinatorial library approach to build sugar inhibitors based on the LacNAc and Lac basic structures have been attempted. We have taken liberty to recalculate from published data, the affinities of various oligosaccharides relative to lactose for galectin-3 (Table 1). Galectin-3 like other members of the family is defined as a beta galactoside binding protein. The addition of a glucose residue to the reducing end of galactose to make lactose (Lac) increases the affinity for galectin-3 considerably [14,84–86]. The exchange of a hydroxyl group in Lac for a more hydrophobic acetamide group to make *N*-acetyllactosamine (LacNAc) increases affinity for galectin-3 by over six fold (Table 1), while the addition of a hydrophilic Gal residue to the 3-hydroxyl group of LacNAc further enhances the affinity for galectin-3 by over 23 fold in comparison to lactose [14]. More interesting substitution (ligands # 8–11; Table 1) developed by introducing hydrophobic groups to the 3-position of Gal residue in LacNAc [15] increase the affinities for galectin-3 by 2-50 fold in comparison to LacNAc. Natural and artificial multivalent ligands however have the highest affinities for galectin-3 relative to lactose. For example 3,6-di-*N*-acetyl-lactosamnyl lactose (ligand # 5; Table 1) as a representative (minimal structure) polylactosamine chain is the best ligand for galectin-3 among the simple oligosaccharides shown in Table 1.

Laminin which is a key member of ECM proteins and a target for galectin-3 is a heterotrimer to which galectin-3 has a high affinity, interacting mainly with polylactosamine residues in the glycoprotein. In a detailed study conducted by Barboni *et al*. [16] the binding affinities of full length galectin-3 and its fragments (where amino acids were deleted from the N-terminal end) to laminin immobilized on a Biocore chip were analyzed. The deletion of 103 A.A. to generate the CRD, resulted in an association constant of 0.05 μM^{-1} in comparison to the whole galectin-3 which had a K_A of 1.05 μM^{-1}. More interestingly, CRD containing additional ~10 amino acids from the collagen-like domain had a 2 fold increase in affinity (K_A 0.1 μM^{-1}). Interestingly, the full length and a fragment comprising AA 94-250 of galectin-3 both had a second additional binding site for laminin (2nd K_A 0.3 μM^{-1} and 2.0 μM^{-1} respectively) [16]. This study was very interesting because it suggested that the collagen-like domain or portions of it can adversely influence the reactivity of the CRD domain. We have reported that the cleavage of galectin-3 by matrix metalloproteinases results in a fragment (~22 kDa), which has ~1.2–2 fold higher affinity for laminin than the full length galectin-3 and postulated that this fragment has the potential to play major physiological role(s) in the extracellular compartment [17,18].

Cell surface and extracellular receptors for galectin-3

On the cell surface, galectin-3 interacts with a growing number of ligands, some of which have been well characterized and are summarized in Table 2. In these interactions, galectin-3 binds to the molecules either in its monovalent form or at high concentration in a multivalent fashion [1,19]. However, the precise physiological events subsequent to these binding interactions in most cases can only be speculated. The binding of galectin-3 to CD-98 on Jurkat cells (T-lymphocytes) leads to a rise in intracellular Ca^{2+} [20] while its interaction with the FcgRII receptor in a variety of cell types results in the downregulation of the IL-5 gene [21]. Galectin-3 at low extracellular doses can trigger superoxide production and enhance the lipopolysaccharide-induced release of interleukin-1 [22,23]. It is also established that galectin-3 can cross-link IgE receptors particularly in basophilic cells to trigger cytotoxicity towards intracellular parasites, degranulation and release of serotonin [24,25]. On human neutrophils, galectin-3 binds mainly to CD66a and CD66b [26]. Galectin-3 can also interact with NCA-160, a surface antigen in human neutrophils to induce an oxidative burst [27].

The second group of cell surface receptors for galectin-3 on the cell surface appear to be more relevant in adhesive rather than signal transduction mechanisms. In neuronal tissues, galectin-3 has strong binding interaction with myelin associated glycoprotein (MAG), laminin, tenascin-R, to a lesser degree cell recognition molecule L1, and neural cell adhesion molecule (NCAM) [28]. Colon cancer cells produce significant quantities of mucins on their surfaces and in the extracellular matrices. These mucins contain polylactosamine chains and have been demonstrated to be a major ligand for the endogenous galectin-3 [29]. Binding of galectin-3 to colon cancer mucin may facilitate the homotypic and heterotypic interactions that are involved in cancer metastasis [30]. Other receptors for galectin-3 on colon carcinomas include carcinoembryonic

Table 2. Ligands for galectin-3

Ligand	*Function associated with galectin-3 binding*	*Reference*
Laminin	Modulates cell/ECM adhesion	[70,76,77]
Collagen IV	Modulates cell/ECM adhesion	[34]
Fibronectin Vitronectin	Modulates cell/ECM adhesion	[64,67]
Hensin	Maintains terminal differentiation of epithelial cells	[39]
Elastin	Promotes adhesion of cells to elastin	[69]
Mac-2BP (gp 90)	Promotes cell/cell and cell/ECM adhesion	[78,79]
Cubilin	Adhesions and endocytosis	[52]
MP20 lens protein	Cell/ECM adhesion	[80]
Colon cancer mucins	Cell/cell and cell/ECM adhesion	[29,31]
Carcino-embrionic antigen (CEA)	Cell/cell and cell/ECM adhesion	[31]
Advanced glycosilation end products (AGE)	Mediates endocytosis of AGE	[49, 81]
$\alpha 1\beta 1$integrins	Modulates cell/ECM adhesion	[34,61]
$\alpha M\beta 1$ (CD11b/18, Mac-1 antigen) (human macrophages)	Modulates cell/ECM adhesion	[33,57]
CD66a/CD66b (human neutrophils)	Induces activation NADH oxidase and adhesion of cells to ECM	[26]
CD98 (Jurkat cells)	Induces uptake of extracellular Ca^{2+} and modulates cell/ ECM adhesion	[20]
$CD4^+/CD8^+$(T lymphocytes)	Inhibits apoptosis and modulates cell/ECM adhesion	[56,82]
FcgRII (human eosinophilic leukemia cells)	Downregulates of IL-5 gene expression	[21]
NCA-160 (human neutrophils)	Induces an oxidative burst in neutrophils	[27]
Neural cell adhesion molecules (NCAM) MAG, L1	Modulates cell/ECM adhesion	[28]
IgE (rat basophilic leukemia cells)	Triggers degranulation and serotonin release	[24,83]
Lamp-1, Lamp-2 (tumor cells)	Modulates cell/cell, cell/ECM adhesion	[31,32]
Lipopolysaccharides (LPS) (bacteria)	Adhesion of pathogen organisms to host ECM and cells	[75]

antigen (CEA) and lysosomal associated membrane proteins (LAMPS) [1,31]. Other important cell surface receptors to which galectin-3 binds, include members of the integrin family such as CDIIb/18 integrin (Mac 1) antigen on mouse macrophages [32,33] and $\alpha_1\beta_1$ [34]. The interactions of galectin-3 with integrins obviously suggest roles in the modulation of cell to cell or cell to extracellular matrix adhesions as discussed below.

Role of galectin-3 in organogenesis

Kidney development

Expression of galectin-3 has been demonstrated in hamster metanephroi [35] and also during human nephrogenesis [36]. More recently, it has been implicated as a modulator of uteric bud branching in organ culture of developing mouse kidney [37]. In the earliest stages of mouse metanephric development E11 to E12, galectin-3 was not detected. Instead it was restricted to the uteric bud derivatives, collecting ducts and urothelium of renal pelvis. Exogenous galectin-3 perturbed branching in E11 and E12 explants. The role of galectin-3 in kidney development has also been studied extensively using MDCK tubulogenesis model system *in vitro* [8]. Addition of a high concentration of exogenous galectin-3 retarded the growth of MDCK cysts formed while addition of galectin-3 blocking antibodies and inhibitors accelerated the growth of these cysts [8]. In this system, it is assumed that galectin-3 mediates strong adhesive forces, which prevent the motility at sites of cell movement and reorganization occurring during the sprouting and tubule formation. On the other hand galectin-3 is presumed to play a key role in the formation of tight junctions between the cells to maintain polarity. This seems to be the case also with normal breast epithelial cells growing in matrigel, where they form well defined cysts while maintaining polarity [38]. Galectin-3 has been directly implicated in terminal differentiation of epithelial cells where it binds to and polymerizes a high molecular weight glycoprotein known as hensin, which maintains polarity in the differentiated state [39]. The normal development of kidneys however requires proper processing and modification of the cell surface receptors for galectin-3 since in mice where this process is impaired, there is malformation of the tubules [40].

The role of galectin-3 in angiogenesis

Apart from its role kidney tuboligenesis, galectin-3 has also been implicated in an vitro model of angiogenesis [41]. Their data demonstrated clearly that proper formation of capillary tubes by endothelial cells *in vitro* requires galectin-3 since the process is blocked by antibodies to galectin-3 and by lactose. Here again it is possible that galectin-3 is involved in

the modulation of weak and strong adhesion between cells and ECM and cell-to-cell interactions. The precise mechanisms involving galectin-3 are yet to be elucidated in this system since a number of parameters are involved. Neovascularization is one of the key processes in tumorigenesis and further studies involving galectin-3 knockout mice will be needed to address the role that galectin-3 plays in this important process. A role has been suggested for galectin-3 in fetal implantation and uteroplacental complex [42] despite the fact that normal implantation has been demonstrated to occur in galectin-3 knockout mice [43]. In such cases, it is possible that galectin-3 is the preferred lectin, but can be substituted by other galectins such as galectin-5.

Galectin-3, endocytosis/exocytosis and signaling

Galectin-3 by virtue of its lack of signal peptide is essentially a cytosolic protein and indeed its intracellular levels far exceed the concentration on the cell surface in most cases. The mechanisms by which it is externalized to the extracellular compartment has yet to be elucidated and may be a novel pathway particularly in tumor cells where its rapid rate of secretion cannot be accounted for by non-classical mechanisms of secretion [44–46]. We recently demonstrated that galectin-3 in the extracellular compartment could be internalized by tumor cells just as rapidly as its exocytosis depending on its extracellular concentration [48]. This process was inhibited by the antibiotic filipin but not chlorpromazine, suggesting internalization via the caveolae membrane microdomains and not clathrin coated pits [47]. More importantly, our studies demonstrated that extracellular galectin-3 at a concentration approaching 100 μg/ml could effectively mediate the endocytosis of beta-1 integrins [34,48]. Further studies in support of our findings suggest that galectin-3 can mediate the endocytosis of advanced glycation end products (AGE) and acetylated-low density lipoproteins (LDL) by CHO cells [49]. Studies in which wild type and galectin-3 knockout mice were induced to manifest diabetes, renal/glomerular AGE accumulation was observed in the galectin-3 null mice but not the wild type [50], suggesting that galectin-3 is a mediator of endocytic degradation of these products by the kidney cells. Accumulation of AGE in the extracellular space is responsible for the deleterious effects of diabetes and so galectin-3 by facilitating the endocytosis of these substances can alleviate the cellular damage associated with the disease. Similarly, galectin-3 mediated endocytosis of acetylated LDL into macrophages and endothelial cells to make foam cells implicates it in atherogenesis [49]. In these studies, galectin-3 is seen more as a facilitator of endocytosis rather that the actual receptor which is directly responsible for the endocytic uptake. To further emphasize this point, cubulin, a 400 kD membrane protein, which has been implicated in the endocytosis of a variety of molecules [51], was recently shown to be associated with galectin-3 [52], suggesting a supportive role of galectin-3 in endocytosis.

The ease with which galectin-3 traverses membrane barriers particularly from the cytosol to the extracellular space despite its lack of signal peptides is pointing to a larger role for this lectin in normal as well as pathophysiology. Galectin-3 may interact with key amphipathic molecules such as synnexin [3] to affect a variety of cellular functions such as exocytosis and endocytosis. There are other multifunctional proteins, which behave like galectin-3 with respect to its ability to cross membrane barriers. Annexins for example are synthesized on free ribosomes and as such residents of the cytoplasm, but can be externalized easily without the help of signal peptides [53]. Just like galectin-3, they are also regarded as multifunctional proteins whose extracellular functions are not precisely known. For example annexin-5 is a marker of apoptosis because it specifically binds to phosphatidyl serine which flip-flops to the external leaflet of the bilayer during apoptosis [54]. The question to be pondered at this juncture is whether galectin-3 is involved in the exocytosis and endocytosis of other key molecules in responsive cells and if so, what are the precise mechanisms in the endocytosis and exocytosis pathways that are mediated by galectin-3? These are clearly questions that need to be addressed for us to have a full appreciation of the repertoire of extracellular functions of gelectin-3.

The presence of galectin-3 in caveolae or lipid rafts [48] suggests that it is a raft organizer, a function that has already been suggested for galectin-4 [87]. Interestingly, a number of signaling molecules are sequestered in these raft domains [88–90]. Both recombinant galectin-8 and -3 have been demonstrated to promote cell spreading [63,64] and integrin dependent signaling [62]. UDP-*N*-acetylglucosamine: a-6D mannoside β1,6 *N*-acetylglucosaminyltransferase V or Mgat5 enzyme produce N-glycan intermediates that are elongated with poly *N*-acetyllactosamine to create ligands for galectin-3. It has been reported that Mgat-modified N-glycans on the T cell receptor (TCR) complex bind to galectin-3, sequestering TCR within a multivalent galectin–glycoprotein lattice that impedes antigen-dependent receptor clustering and signal transduction [91,92]. A deficiency in Mgat lowers T-cell activation thresholds by directly enhancing TCR clustering. Consequently Mgat deficient mice show kidney autoimmune disease, enhanced delayed-type hypersensitivity, and increased susceptibility to experimental autoimmune encephalomyelitis. Similarly integrin receptor, and for that matter other cell surface molecules which contain polylactosamine residues, and which are involved in signaling are also sensitive to changes in Mgat5-dependent N-glycosylation. The studies suggest that low affinity but high avidity interations between N-glycans and galectins can regulate the distribution of cell surface receptors and their responsiveness to agonists [92].

Galectin-3 and cellular adhesion

The interaction of cells with the extracellular matrix proteins is essentially mediated by integrins [55]. There are, however, a number of proteins on the cell surface, which modulate the activities of integrins. In an adhesion model proposed by Hughes

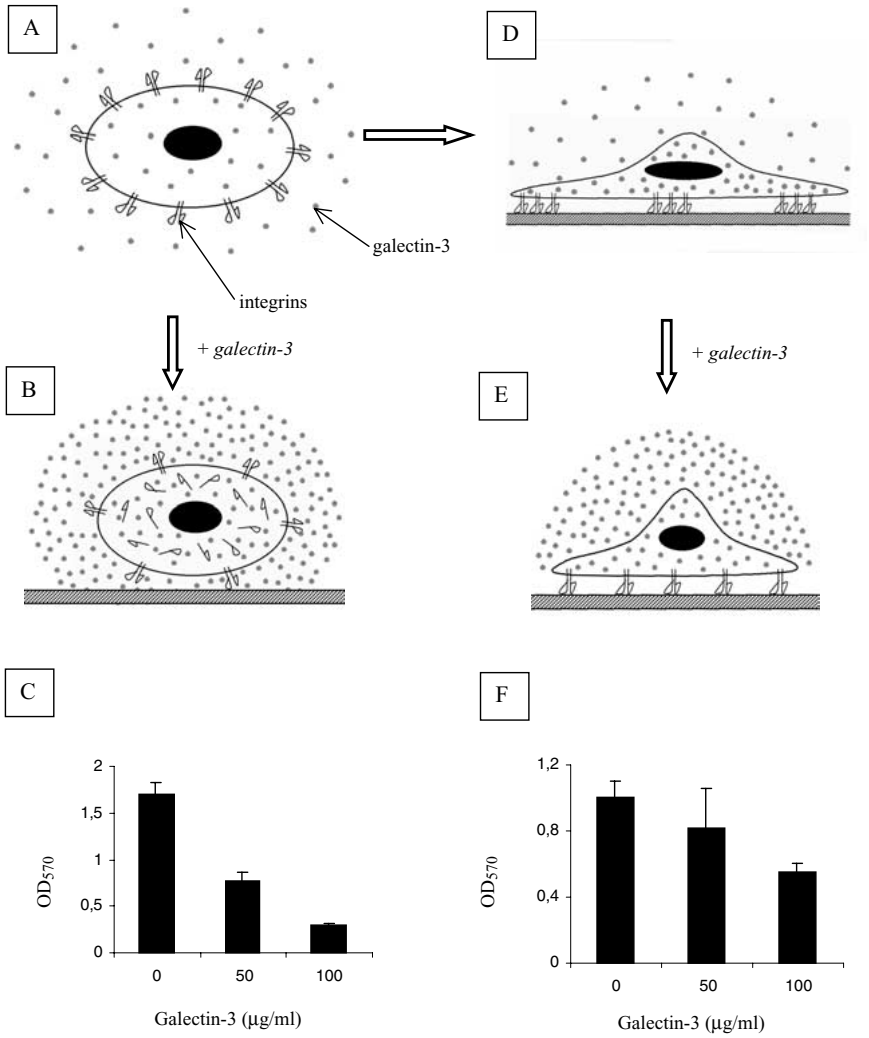

Figure 1. A model depicting the regulation of MDA-MB-435 breast carcinoma cell adhesion to collagen IV by galectin-3. If the cells in panel A are incubated with 100 μg/ml of recombinant galectin-3 for 30 min at 37°C and then allowed to adhere to the wells of a micro-titer plate coated with collage IV (10 μg/well) and blocked with 2% BSA, the cells hardly adhere to the wells (panel B) and as illustrated by the dose response curve in panel C. If on the other hand, the cells in panel A are allowed to adhere to the wells of a micro-titer plate coated with collagen IV and after attachment and spreading (panel D), galectin-3 added to a concentration of 100 μg/ml, the adhered cells are only partially detached (panel E). This is also illustrated by a dose response curve in panel F.

[56], galectin-3 can ligate the glycosylated cell surface CD-98 which in turn can mediate integrin clustering on the surface of cells to increase avidity of binding [56]. We and others have shown that $\alpha_1\beta_1$, and CDIIb/18 integrin can also interact directly with galectin-3 [34,57]. Galectin-3 is likely to bind via its CRD to the tri and tetra antennary branches of polylactosamine residues on the integrins [58,59]. The binding interaction is then presumed to either alter the affinities or by steric hindrance negate the binding of integrins to their ECM ligands. To study this phenomenon much further, we have demonstrated that liposomes reconstituted with $\alpha_1\beta_1$ integrins have reduced interaction with laminin or collagen in the presence of galectin-3 and that the purified integrins can directly interact with galectin-3 (unpublished information).

Post-translational modification of integrins with respect to polylactosamine residues is usually enhanced after cellular transformation and may increase further with tumor progression [60]. According to the model depicted in Figure 1, the regulation of the adhesion of tumor cells to ECM proteins by galectin-3 is largely a function of its extracellular concentration. At low to moderate *in vivo* extracellular concentrations (0.1–0.25 μM) of galectin-3, normal adhesion of tumor cells to ECM is achieved (Figure 1D). At these levels, galectin-3 may even stimulate adhesion of cells to the ECM components either directly or indirectly by clustering the integrin molecules in discrete membrane microdomains thereby increasing avidity of binding as described above [77]. In the presence of high extracellular concentration of galectin-3 particularly when

non-adherent tumor cells are incubated with ~5 μM of galectin-3, the lectin binds to integrins particularly of the VLA family to reduce their interaction with ECM ligands and to sequester them inside the cells [34,48]. The overall effect is that the integrins are not available for adhesion and so the cells remain detached (Figure 1B and C). It is not known whether such a high concentration of galectin-3 can be achieved *in vivo* but it is possible that in certain microdomains on the cell surface, galectin-3 concentration can be high enough as to negate the interaction of integrins with their ECM ligands.

If on the other hand galectin-3 is added to the cells which are already adhered to the ECM proteins (Figure 1D), the inhibition of adhesion is not as dramatic (Figure 1E and F). This is most likely due to the fact that the integrins are already engaged to their ECM ligands and the added galectin-3 may only reduce their affinities for the ECM without stimulating their endocytic uptake from the cell surface.

Galectin-3 is therefore postulated to modulate the adhesion of all cell types whose integrins are appropriately glycosylated to the ECM [58,61]. When the integrins are only marginally glycosylated as would be expected in normal epithelial cells or fibroblasts, then cell to extracellular matrix interactions may be controlled by other integrin accessory proteins and as such may not be responsive to galectin-3. Therefore the tight regulation of tumor cell adhesion by galectin-3 is one, which can be an interesting therapeutic target *in vivo*. In normal epithelial cells, galectin-3 is likely to play a major role in the maintenance of the differentiated phenotype rather than the interaction of cells with ECM proteins as alluded to above. Galectin-3 in normal differentiated epithelium is usually secreted towards the apical surface (lumen) rather than the basal surface [44].

Having considered a scenario where galectin-3 inhibits the adhesion of responsive cells to their ECM ligands, is it then correct to proclaim that galectin-3 is an anti-adhesive protein in these cell systems? On the contrary, based largely on indirect data, and depending on the cell type or its extracellular concentration, galectin-3 like galectin-8 can be considered to function as a matricellular protein which can be both pro- and anti-adhesive [62]. Breast carcinoma cells with high expression of galectin-3 such as MDA-MB-435 and MDA-MB-231 interact well and spread very rapidly on ECM proteins compared to those with low or no expression of galectin-3 such as BT-549 and SK-Br-3 [63,64]. Moreover it was recently demonstrated that downregulation of galectin-3 in MDA-MB-435 by the antisense approach gave rise to a subclone which spreads poorly, grows poorly in soft agar and forms tiny tumors in nude mice compared to the cells transfected with the empty vector [65]. Galectin-3 clearly is important in the interactions of these tumor cells with their ECM ligands, and appears to use a novel mechanism to promote the adhesion and spreading of these cells to their extracellular matrices. Galectin-3 may accelerate the secretion and expression of integrins on the cell surface [63,64] and secretion/exocytosis of collagen [66] to the extracellular compartment to enhance adhesion and spreading. Galectin-3 can also ligate the cells to ECM ligands such as laminin and fibronectin even though this may require higher concentrations of the lectin.

The major extracellular matrix proteins laminin and fibronectin contain polylactosamine residues to which galectin-3 can bind [67]. High extracellular concentrations (2.5–5 μM) of galectin-3 using the positive cooperativity phenomenon [13,19,68] can theoretically ligate cells to these proteins in a divalent independent manner. We have demonstrated that galectin-3 is essential for the interaction of breast carcinoma cells with elastin fibers [69]. Galectin-3 itself may be immobilized in the extracellular matrix [70] and cells can in turn bind directly to it in a carbohydrate dependent manner [71]. In bacteria and lower eukaryotes, galectin-3 is increasingly becoming a major player in their adhesion to ECM proteins and host tissues. We recently demonstrated that T. cruzi trypomastigotes can be ligated directly to laminin by galectin-3 in a lactose dependent manner [72]. Galectin-3 can bind to lipopolysaccharides on bacteria to mediate their interaction with host cells and extracellular matrices [73–75].

Conclusions

Shortly after its discovery galectin-3 was thought to play largely a cell to extracellular matrix adhesive role. Now a plethora of physiological functions in the extracellular compartment have been assigned to it and more are yet to be discovered. Because of its chimeric nature, galectin-3 can be assumed to have more extracellular interacting partners than its brethren, which display only the carbohydrate recognition domains. For example a number of non-carbohydrate binding activities have recently been observed in the interactions in which galectin-3 is involved. The ability of galectin-3 to polymerize hensin in the maintenance of epithelial differentiation and polarity could not be inhibited by lactose or other saccharides [39], suggesting a protein-protein interaction. Another noted example is the interaction of galectins with the bacterial endotoxin lipopolysaccharide (LPS). Two independent LPS binding sites on galectin-3 have been demonstrated. One is on the CRD and is responsible for the binding of LPS from Klebsiella pneumoniae which has a beta-galactoside containing polysaccharide chain [73]. In sharp contrast, LPS from Salmonella minessota R7 appears to bind to the N-terminal region of galectin-3 since the binding interaction cannot be inhibited by lactose [73]. We have reported a direct interaction of galectin-3 with insoluble elastin even though this extracellular matrix protein lacks sugar residues [69]. We have demonstrated that galectin-3 interacts with the chemotactic (VGVAPG) domain of elastin (unpublished information), demonstrating that this is another fine example of protein/protein interaction involving galectin-3.

Future research directions in galectin-3 research will undoubtedly focus on its role in the pathological conditions such as diabetes, cardiovascular, tumor metastasis and autoimmune diseases. Unique mechanisms that galectin-3 may be involved

in these pathological conditions would be prime targets for therapeutic interventions.

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