Review

Galectins: matricellular glycan-binding proteins linking cell adhesion, migration, and survival

M. T. Elola[a], C. Wolfenstein-Todel[a], M. F. Troncoso[a], G. R. Vasta[b] and G. A. Rabinovich[c, *

[a] Instituto de Química y Fisicoquímica Biológicas (IQUIFIB), Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956 (C1123), Buenos Aires (Argentina)
[b] Center of Marine Biotechnology, University of Maryland Biotechnology Institute, 701 East Pratt Street, Baltimore, Maryland 21202 (USA)
[c] Instituto de Biología y Medicina Experimental IBYME, CONICET, Vuelta de Obligado 2490 (C1428) Buenos Aires (Argentina), Fax: +54 11 4786 2564, e-mail: gabyrabi@ciudad.com.ar

Abstract. Galectins are a taxonomically widespread family of glycan-binding proteins, defined by at least one conserved carbohydrate-recognition domain with a canonical amino acid sequence and affinity for β-galactosides. Because of their anti-adhesive as well as pro-adhesive extracellular functions, galectins appear to be a novel class of adhesion-modulating proteins collectively known as matricellular proteins (which include thrombospondin, SPARC, tenascin, hevin, and disintegrins). Accordingly, galectins can display de-adhesive effects when presented as soluble proteins to cells in a strong adhesive state. In this context, the de-adhesive properties of galectins should be considered as physiologically relevant as the pro-adhesive effects of these glycan-binding proteins. This article focuses on the roles of mammalian galectins in cell adhesion, spreading, and migration, and the cross-regulation of these functions. Although careful attention should be paid when examining individual galectin functions due to overlapping distributions, these intriguing glycan-binding proteins offer promising possibilities for the treatment and intervention of a wide variety of pathological processes, including cancer, inflammation, and autoimmunity.

Keywords. Galectins, adhesion, de-adhesion, spreading, migration, inflammation, immunity.

Introduction

Galectins (Gal) are a ubiquitous, ancient family of carbohydrate-binding proteins defined by at least one conserved carbohydrate-recognition domain (CRD) with a canonical amino acid sequence, and affinity for β-galactosides. Galectins are present in vertebrates, protochordates, invertebrates, mushrooms such as *Agrocybe cylindracea* and *Coprinus cinereus*, and viruses [1, 2].

To date, 15 distinct galectins have been identified in mammals. However, the inclusion of Gal-11, which was first characterized as a lens-specific protein called GRIFIN (*galectin-related interfiber protein*) remains controversial. In fact, Gal-11 lacks two of the seven key amino acid residues conserved in most galectin CRDs and did not display β-galactoside-binding activity even when these two residues were mutated to the conserved motif [1, 3]. According to their structure, galectins have been classified as: a) mono-

---

* Corresponding author.

valent galectins, containing a single CRD, that may form homodimers to become functionally bivalent; b) bivalent tandem-repeat galectins possessing two CRDs; and c) chimeric galectins with a single CRD and a unique amino terminus [4]. Gal-1, -2, -5, -7, -10, -13, -14, and -15 are composed of a CRD and a short N-terminal sequence (prototype galectins). Gal-4, -6, -8, -9, and -12 have two non-identical CRDs in tandem with a short linker sequence (tandem-repeat type galectins), and are most likely derived from an ancestral duplication of a single-CRD galectin [5]. Gal-3 is the only chimeric galectin found to date, and possesses one CRD (COOH-terminal domain), an extended R domain consisting of glycine/proline repeats, and a short NH_2-terminal end (N domain).

Galectins recognize glycoconjugates that contain $Gal\beta1,4GlcNAc$ (LacNAc) sequences that can be present on N- or O-linked glycans [6, 7]. In the intracellular milieu, galectins bind to their ligands preferentially through protein-protein interactions, and regulate intracellular processes, including pre-messenger RNA (mRNA) splicing, cell cycle progression, apoptosis, and cell proliferation [8].

Galectins lack a signal peptide, and are secreted to the extracellular milieu through a non-classical endoplasmic reticulum (ER)/Golgi-independent pathway [9, 10]. Therefore, the efficiency of galectin export is not affected by brefeldin A, a drug that blocks ER/Golgi apparatus-dependent secretory transport. Compelling evidence indicates that the galectin secretion mechanism involves the formation of exovesicles generated by membrane blebbing [9]. Gal-1 secretion was initially described by Cooper and Barondes [11] in skeletal muscle cells, although the molecular mechanisms involved in this secretory pathway remained elusive for many years. Recently, Nickel's group has demonstrated that secretion of Gal-1 from mammalian cells depends on functional interactions between the lectin and its counter-receptors [12]. Thus, single-site mutations in Gal-1 caused both counter-receptor binding and export deficiencies in CHO cells. Moreover, wild-type Gal-1 failed to export from a CHO mutant cell line defective in a Golgi apparatus-resident UDP-galactose transporter. Therefore, functional interactions with counter-receptors seem to be essential for the overall Gal-1 export process.

Two models have been proposed by Nickel for this non-conventional secretory pathway. First, β-galactoside-containing cell surface molecules might be tightly coupled to the translocation machinery. Thus, counter-receptors may function by exerting a pulling force at the extracellular side of the putative translocation pore required for directional transport of Gal-1 across the plasma membrane. In a second model, counter-receptors could act as export receptors for Gal-1: i.e.

β-galactoside-containing glycolipids from the extracellular leaflet of the plasma membrane may be translocated to the inner leaflet, this process catalyzed by a plasma membrane resident enzyme. Subsequently, retrotranslocation of counter-receptors occupied by Gal-1 would mediate export to the extracellular space.

Although both models are speculative, they are consistent with experimental results demonstrating Gal-1 translocation at the level of the plasma membrane [9–11, 13]. Further, pharmacological evidence showed that Gal-4 secretion is impaired in epithelial cells following treatment with an inhibitor of glycosylation [14].

In the case of Gal-3, Ochieng's group demonstrated that Gal-3 may be able to interact directly with membrane lipids in solid-phase assays, and spontaneously penetrate the lipid bilayer of liposomes in either direction, in an energy-independent manner [15]. A unique stretch of sequence of the Gal-3 N-terminus domain may be determinant for secretion [16], although Gal-3-containing exosomes have also been described [10]. In summary, secretory processes of different galectins may involve both non-vesicular and vesicular pathways. Further studies are required to identify membrane translocation-specific components of the export machinery.

Although the generally held view is that galectins are soluble proteins secreted to the extracellular milieu, the urate channel/transporter (UAT), which was later designated as Gal-9, is a clear exception [17]. Computer modeling and lipid bilayer studies of the urate channel/transporter predicted a molecular model containing four transmembrane domains, two extracellular β-galactoside-binding sites, and the intracellular amino and carboxy termini; the unique linker portion comprises two transmembrane domains and the intracytoplasmic loop [17]. Therefore, for the first time, membrane targeting was demonstrated for a galectin family member, although cytosolic and secreted forms of Gal-9 have also been described [18].

A wide range of biological functions have been described for galectins, including regulation of cell adhesion, migration, cell growth, apoptosis, and pre-mRNA splicing. Extracellularly, they bind to β-galactoside-containing glyconjugates of extracellular matrix (ECM) components and cell surface adhesion molecules. Interestingly, research over the past decade has identified a novel role for galectins as versatile regulators of cell-cell and cell-matrix interactions. Galectins display the capacity to act as biological cross-linkers for ECM proteins and cell surface receptors, implicating galectins as a novel class of matricellular proteins that modulate cellular interactions [19, 20] (Fig. 1). Examples of matricellular

proteins that serve as adapters between cells and the ECM are thrombospondin, tenascin, SPARC, osteopontin, and hensin. Thrombospondin-1, for example, stimulates the loss of focal adhesions and stress fibers in spread adherent bovine aortic endothelial cells plated on fibronectin (FN).

Several biochemical and functional properties of galectins fit with those features of matricellular proteins: 1) they do not contribute to structural roles in the ECM but function contextually as modulators of cell-matrix interactions; 2) they bind to many cell surface receptors, ECM proteins, cytokines, and proteases; 3) they function as both soluble and insoluble (substrate) proteins in the ECM; 4) they show de-adhesive as well as adhesive properties; 5) targeted deletion of galectin genes does not affect embryogenesis, suggesting redundancy of biological functions (although some abnormalities are present) [19].

This review article focuses on the agonistic/antagonistic roles of mammalian galectins in cell adhesion, spreading, and migration as strongly regulated and interconnected functions. These processes are required for survival of normal anchorage-dependent cells. Moreover, attached non-transformed cells, which cannot spread, undergo a particular process of homeless-induced apoptosis called anoikis which is caused by loss of cell anchorage and can be prevented by laminin (LN), FN, and Gal-3 [7, 21, 22]. Thus, a deeper understanding of the pleiotropic functions of this evolutionarily conserved protein family should contribute significantly to address fundamental questions in cell biology.

Galectin-1

Galectin-1 in cell-substrate adhesion

Cell adhesion is a complex process that occurs in three stages: attachment, spreading, and formation of focal adhesions and actin-containing stress fibers. Cell spreading and rearrangements of the cytoskeleton are required for survival of normal, non-transformed cells. Gal-1 shows both pro-adhesive and anti-adhesive functions, as it can potentiate or inhibit cell-ECM and cell-cell interactions. Several adhesion studies were performed using Gal-1 as a substrate. Initial reports demonstrated that Gal-1 contained in ECM from bovine corneal endothelial cells (ECs) promoted the adhesion of A121 ovarian carcinoma cells to matrix [23] (Table 1). Adhesion to Gal-1-coated plates has been demonstrated by several authors (Fig. 1b). Ahmed et al. [24] performed adhesion assays with B142 lymphoblastoid cells, coating plates with Gal-1, either in its dimeric or polymerized state, and found

that the dimeric lectin was unable to promote adhesion up to a concentration of 100 µg/well, whereas the polymerized lectin was effective at a concentration of 0.6 µg/well. Similarly, Skrincosky et al. [25] found a significant increase in the lactose-inhibitable adhesion of A121 ovarian carcinoma cells to polymerized Gal-1-coated plates, while only 37% of the cells adhered to adsorbed dimeric Gal-1. Further, the adhesion of splenocytes to plates coated with sheep spleen Gal-1 has been reported [26].

Gal-1 promotes the adhesion of normal and cancer cells to ECM glycoproteins such as LN [27–29]. Zhou and Cummings [27] reported that Gal-1 from porcine heart promoted the Ca^{2+}-independent adhesion of CHO and F9 cells to LN, in a lactose-inhibitable manner. Similarly, Mahanthappa et al. [28] demonstrated that Gal-1 mediates adhesion of olfactory rat neurons to LN-coated coverslips; the number of adherent cells increased as the amount of substrate-bound Gal-1 increased, and thiodigalactoside (TDG), a non-metabolizable competitive inhibitor of Gal-1, completely inhibited the lectin effects. Given these observations, it was hypothesized that Gal-1 could mediate *in vivo* olfactory axon fasciculation by cross-linking adjacent axons and promote axonal adhesion to the ECM.

In addition, Gal-1 has been shown to enhance A375 and A2058 human melanoma cell adhesion to LN. Adhesion assays have shown that recombinant Gal-1 (rGal-1) increased melanoma cell attachment to LN in a dose-dependent manner (Fig. 1a), while anti-Gal-1 antibodies significantly decreased Gal-1-induced stimulation of cell attachment. Therefore, it is likely that local increase or decrease of Gal-1 expression in the tumor microenvironment may play a critical role during attachment and detachment of cancer cells throughout cancer progression [29].

Moreover, in human ovary carcinoma, increasing concentrations of rGal-1 induced a dose-dependent increase in adhesion to LN-1 in AZ224 and AZ382 cells, as well as an increase in OVCAR-3 cell adhesion to FN. Therefore, Gal-1 can modulate interactions between LN-1/FN and glycoconjugates at the cell surface [30]. In certain cases, both adhesion and apoptosis of tumor cells were modulated by Gal-1. For example, Gal-1-transfected colon cancer Colo201 cells showed a significantly higher number of adherent cells cultured on FN-, LN-, and collagen-coated dishes when compared to mock transfected cells. In addition, apoptosis of the transfected tumor cells was also observed [31].

In contrast to these findings, negative regulation of cell adhesion to LN/FN substrates by Gal-1 has also been well documented. These results are in accordance with the de-adhesion model postulated for
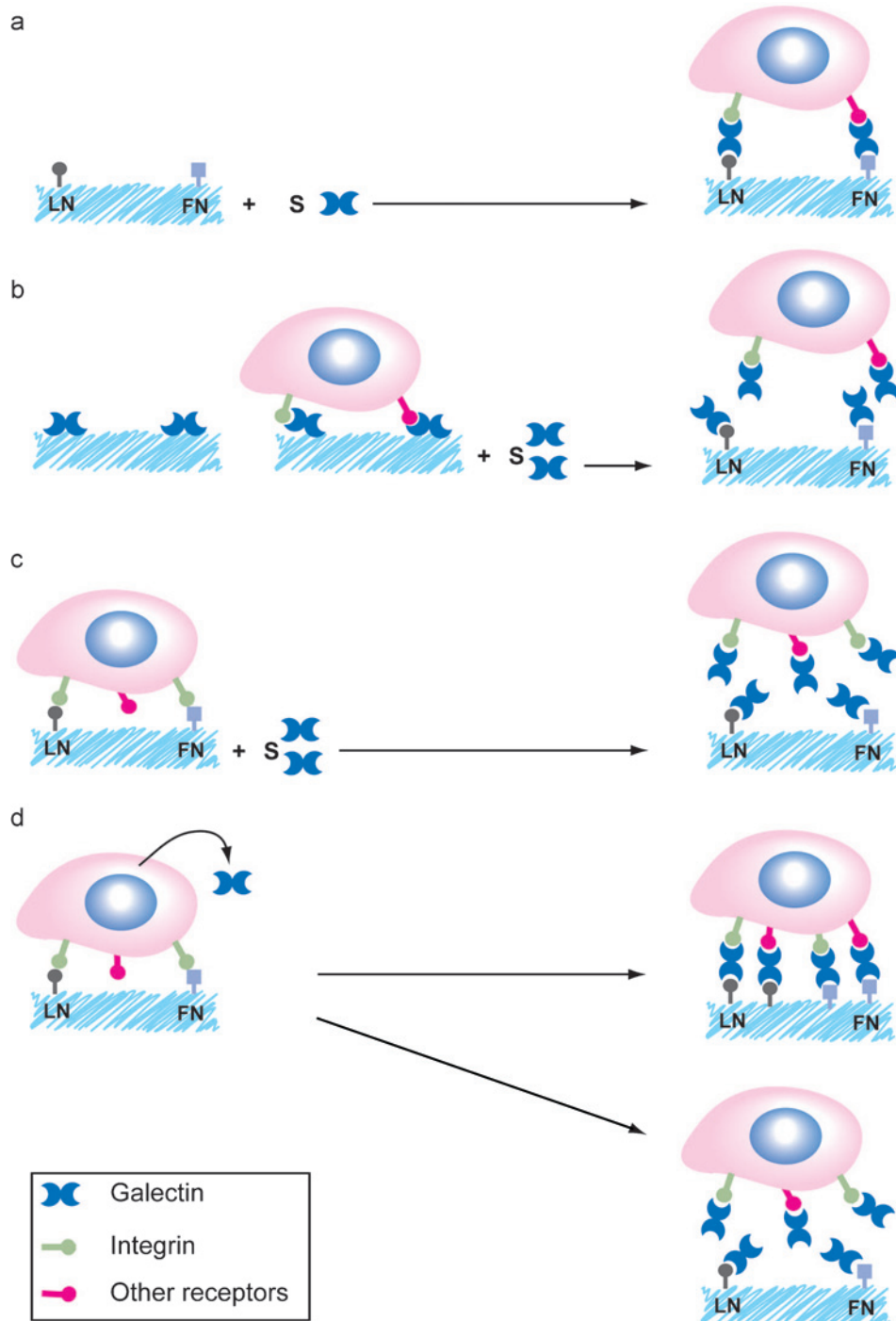
Figure 1. Model of cell to substrate adhesion mediated by galectins. (a) Low concentrations of soluble (S) galectins promote adhesion of cells to extracellular matrix proteins such as laminin (LN) and fibronectin (FN) by bridging different galectin cell membrane receptors and LN/FN. (b) Immobilized galectins promote cell adhesion by crosslinking cell membrane galectin receptors. Addition of high concentrations of the S galectin to cells already adhered to immobilized galectin promotes de-adhesion by interacting with receptors. (c) When cells are adhered to LN or FN as substrates, high concentrations of the S galectin promote de-adhesion. In fact, the galectin binds to both LN/FN and galectin cell membrane receptors such as integrins, which are themselves receptors for LN/FN. (d) Cells engineered to overexpress a certain galectin secrete the lectin to the extracellular milieu. Two different models have been proposed, probably depending on the galectin type, different levels of galectin secretion, the cell type, and distinct cell membrane receptors for each galectin. Thus, galectin can promote cell adhesion to LN or FN (by bridging galectin cell membrane receptors and LN/FN). On the other hand, the secreted galectin can induce de-adhesion by interacting with both LN/FN and integrins. The model has been simplified, considering that only one type of galectin and only two different cell membrane galectin receptors are present. Other galectin cell membrane receptors: lysosome-associated membrane proteins 1 and 2 (LAMP-1 and LAMP-2), Thomsen-Friedenreich antigen (TFAg), glycoprotein 90K/Mac-2BP, cancer antigen CA125, carcinoembryonic antigen (CEA), GM1 ganglioside, etc. [6].

Table 1. Cell adhesion mediated by galectins.

Probe		Cell type	Adhesion	Ref.
Gal-1	immobilized	A121 human ovarian carcinoma	ECM (Gal-1 containing)↑	23
		A121 human ovarian carcinoma	Gal-1 coating↑	25
		B142 lymphoblastoid	polymerized-Gal-1 coating↑	24
		sheep splenocytes	Gal-1 coating↑	26
		rat olfactory neurons	LN↑	28
	soluble	CHO, F9 teratocarcinoma	LN↑	27
		A375, A2058 human melanoma	LN↑	29
		AZ224, AZ382 human ovarian carcinoma	LN-1↑	30
		OVCARC-3 human ovarian carcinoma	FN↑	30
		C2C12 murine myoblast	LN↓	32
		IL-2-activated human T-cells	LN, FN, ECM↓	34
		human vascular smooth muscle	LN↓	36
		rat Leydig	uncoated plates↓	37
		various murine and human tumor cells	LN, FN↓	38
		A375 human melanoma	homotypic↑	39, 40
	endogenous	murine RAW117-H10 large-cell lymphoma	murine endothelial cells↑	41
		MDA-MB-435 human breast carcinoma	human endothelial cells↑	42
		MOLT-4 human T lymphoblastoid	human thymic epithelial cells↑	43
			human thymocytes↑	
	transfected	Colo201 human colon cancer	FN, LN, collagen↑	31
Gal-2	soluble	human T-cells	collagen I↓	64
			FN↑	
Gal-3	soluble	human neutrophils	LN↑	66
		XK4-A3 human breast adenocarcinoma	LN↑	73
		11-9-1-4 human breast carcinoma, HT-1080 human fibrosarcoma, PC-3 human prostate carcinoma	LN, FN, collagen IV↓	76
		murine thymocytes	murine thymic epithelial cells↓	99
		A375 human melanoma	homotypic↑	81
	endogenous	MDA-MB-435 human breast and DU-145 human prostate carcinomas	homotypic (aggregation)↑	42, 79, 80, 82
		murine dendritic cells	human endothelial cells↑	
		murine lung vascular endothelial cells	murine lymphocytes↑	92
			murine melanoma cells↑	87
	transfected	sf9 insect	homotypic↑	78
		BT-549 human breast carcinoma	LN, collagen IV↑	69
		Evsa-T human breast carcinoma	LN, FN, vitronectin↑	22
	anti-Gal-3	PC-3 human prostate carcinoma	human endothelial cells↓	85
		MDA-MB-435 human breast carcinoma	human endothelial cells↓	86
Gal-4	immobilized	T84 human colon cancer	Gal-4 coating↑	109
Gal-8	immobilized	HeLa, CHO, NIH-hIR	Gal-8 coating↑	125
		human neutrophils	Gal-8 coating↑	133
		human Jurkat T-cells	Gal-8 coating↑	134
	soluble	HeLa, CHO-P, HaCaT	uncoated plates↓	125
		HeLa human cervix carcinoma	LN, FN↓	125
		1299 human lung carcinoma	uncoated plates↓	118
		human neutrophils	uncoated plates↑	129
Gal-9	immobilized	human eosinophils	Gal-9 coating↑	137
	soluble	human eosinophils	homotypic (aggregation)↑	142
		MM-RU human melanoma	homotypic (aggregation)↑	143
		mouse Th1 lymphocytes	homotypic (aggregation)↑	144
	endogenous	human eosinophils	activated human endothelial cells↑	140
	transfected	MCF-7 human breast carcinoma	LN, vitronectin↓ FN, collagen IV↓ homotypic (aggregation)↑	138
		Ca9-22 human oral carcinoma	FN, collagen I↑	139
	anti-Gal-9	Eol-1 human eosinophilic leukemia	activated human endothelial cells↓	141
		human eosinophils	HFL-1 human lung fibroblasts↓	137

ECM: extracellular matrix; LN: laminin; FN: fibronectin; Anti-Gal: anti-galectin antibody.

matricellular proteins when the latter are presented as soluble proteins to cells strongly adhered to a substrate. Elegant studies by Barondes' group clearly demonstrated that the addition of soluble Gal-1 to differentiating C2C12 mouse myoblasts plated on LN inhibits cell adhesion and spreading (Fig. 1 c). This effect is carbohydrate-dependent since TDG almost completely blocks this inhibitory effect. Moreover, when myoblasts are stably transfected with an expression vector engineered to constitutively secrete Gal-1, cells also show defects in adhesion and spreading on LN. Besides, pre-digestion of LN with glycosidases blocks Gal-1-induced inhibition of cell adhesion, demonstrating that Gal-1 binding to LN, and not to the cell surface, mediates detachment [32]. In fact, myoblast-derived Gal-1 binds to both LN and to $\alpha_7\beta_1$ integrin, the prominent LN-binding integrin on differentiating skeletal muscle cells, and can effectively inhibit the association between LN and this integrin [33].

Similarly, exogenous Gal-1 inhibited interleukin-2 (IL-2)-induced T-cell adhesion to ECM glycoproteins [34]. Adding rGal-1 substantially decreased the adhesion of activated T-cells to whole ECM or intact LN or FN in a dose-dependent manner (Fig. 1 c). Moreover, an antibody against Gal-1 almost completely abrogated the anti-adhesive effect of the lectin on the different ECM components, while TDG partially abolished T-cell attachment. In addition, Gal-1 prevented the actin cytoskeleton-mediated spreading of IL-2- or phorbol-myristate acetate (PMA)-activated T-cells.

Furthermore, Gal-1 and plasma FN were equally suitable as attachment substrates for smooth muscle cells (SMCs), and the binding to Gal-1 was strikingly reduced by lactose [35]. Soluble Gal-1 inhibited the attachment of SMC to LN in a dose-dependent manner, and Gal-1 also inhibited SMC spreading on LN through binding to both $\alpha_1\beta_1$ integrin on SMC and to LN [36] (Fig. 1 c).

In rat Leydig cells, addition of Gal-1 (14 μM) caused a 70% decrease of cell adhesion onto uncoated plates compared with basal values. Simultaneous incubation of these cells with Gal-1 and lactose prevented this effect. When cells were cultured on LN-1-coated plates, enhanced adhesion was observed, and cells were protected from Gal-1-induced detachment [37]. Tumor cell adhesion to LN was also evaluated by André et al. [38], using galectin-saturated cells and/or matrix. The authors found that Gal-1 saturation of reactive sites on the cell surface reduced adhesion more effectively than saturation of LN with this lectin. Therefore, an increase in cell surface Gal-1 induced by experimental manipulations led to reduced binding of various tumor cells. These effects might be associated

with competitive inhibition of critical cell surface binding sites (Fig. 1 c). Although these results led to the conclusion that immobilized Gal-1 promotes cell adhesion, experiments performed with soluble Gal-1 showed contradictory results. While in some situations enhanced cell adhesion was observed, in other cases Gal-1 had the opposite effect. These differences could be attributed to: a) different cell-membrane receptors for Gal-1 according to the cell type (a distinct glycoconjugate ligand); b) distinct cell-membrane receptors in each cell type for FN or LN, i.e. not only the repertoire of integrins, but other non-galectin cell-surface ligands, which may indirectly influence galectin binding; c) co-expression in the same cell type of Gal-1 and other galectins which could exert opposite adhesive and/or apoptotic effects; or d) variations in galectin concentration and oligomerization state.

Galectin-1 in cell-cell adhesion

Gal-1 has been demonstrated to induce homotypic aggregation in different cell types. For example, in A375 human melanoma cells Gal-1 mediated homotypic cell aggregation, at least in part through binding to the glycoprotein 90K/Mac-2 binding protein (90K/Mac-2BP), an oligomer which can bind various molecules of Gal-1 and Gal-3. This effect was specifically blocked by lactose or by a monoclonal antibody (mAb) against 90K/Mac-2BP. Two possible mechanisms have been postulated to explain this effect: (1) because of the bivalent nature of Gal-1, it might bind to 90K/Mac-2BP displayed on the cell surface of different cells; (2) Gal-1 might interact simultaneously with 90K/Mac-2BP and other cell-surface glycoconjugates from different cells [39]. Interestingly, homotypic cell aggregation mediated by Gal-1 has recently been demonstrated to be inhibited by synthetic lactulose amines [40].

The possible role of Gal-1 in heterotypic adhesion of tumor cells to vascular endothelium has been proposed by several authors as a crucial step related to tumor cell invasion and metastasis. For example, Lotan et al. [41] (Table 1) showed that Gal-1 is expressed in mouse hepatic sinusoidal EC, and that murine RAW117-H10 large-cell lymphoma cell adhesion to hepatic sinusoidal EC or lung microvessel EC was inhibited by anti-Gal-1 antibodies. Similarly, in the adhesion of MDA-MB-435 cells to human EC, accumulation of Gal-1 was detected at the contact sites between breast tumor cells and the endothelium [42].

Gal-1 also mediated the adhesion of MOLT-4 lymphoblastoid cells to thymic epithelial cells, an interaction that could be specifically blocked by anti-Gal-1 antibodies, and by β-galactoside-related sugars. Gal-1 has been shown to be expressed in thymic epithelial

cells, but not in MOLT-4 cells. In addition, antibodies to CD43 and CD45, two well-known T-cell surface glycoprotein ligands, inhibited the binding of MOLT-4 cells to immature cortical thymocytes [43]. These results suggested that Gal-1 participates in thymocyte-thymic epithelial cell interactions during positive or negative selection, showing a potential role for this protein in the generation of central immune tolerance. Taken together, these results suggest that Gal-1 can have a positive effect on cell-cell interactions.

Galectin-1 in cell migration
An important role of different galectins is associated with the regulation of cell migration and invasiveness, which are major hallmarks in tumor progression, i.e. in metastatic processes. Cell migration occurs through multiple adhesion and spreading events: (1) initial cell adhesion to numerous components of ECM with modifications of the ECM molecular composition; (2) cell motility, which involves the reorganization of the actin cytoskeleton, mainly through modifications of integrin-ECM interactions; and (3) invasion, which involves the degradation of ECM proteins by tumor-secreted proteolytic enzymes (serine proteases, cathepsins, and metalloproteinases (MMP) such as MMP-2, MMP-9 and MMP-14) [44].

Different reports described the involvement of Gal-1 in cell migration and chemotaxis. For instance, Gal-1 affected SMC migration in cell culture by interacting with integrins and ECM proteins such as LN and cellular FN. In brief, membranes from transwell chambers were precoated with Gal-1 or LN, and SMC migration was induced by platelet-derived growth factor (PDGF) placed in the lower compartment. Interestingly, SMC migration was higher in Gal-1- than in LN-coated chambers. To study whether Gal-1 affected SMC migration on FN or LN, membranes precoated with these glycoproteins were treated with Gal-1 on the upper surface. The results were clear: precoating with Gal-1 on the upper side resulted in 30% inhibition of SMC migration on FN, but significantly increased the migration of these cells on LN. The inhibitory effect of Gal-1 might be caused by steric hindrance of interactions between ECM proteins and cellular receptors in the presence of Gal-1 [36].

Kiss and colleagues investigated the cell migration properties of Gal-1 in gliomas and colon cancer cells. The authors demonstrated that Gal-1 significantly enhanced the *in vitro* migratory capacity of human U87 glioblastoma cells in a lactose-inhibitable manner, and increased the amount of polymerized filamentous actin. Furthermore, microinjection of Gal-1 antisense oligonucleotides in U87 glioblastoma cells induced a significant decrease (~20%) in the motility

of these cells as compared to controls [45, 46] (Table 2). Similarly, this group [47] performed complementary DNA (cDNA) microarray analysis of U87 tumor astrocytes which were stably transfected with antisense *gal-1* mRNA and found alterations mainly in the quantitative expression of proteins involved in actin polymerization. Interestingly, Maeda et al. [48] demonstrated that Gal-1, but not Gal-3, enhanced the migratory activity of hepatic stellate cells (liver-specific pericytes), which are usually mobilized when the liver is injured and migrate to sites of necrosis to accumulate and exert their functions. In addition, in Schwann cells, oxidized Gal-1 stimulated migration from both the proximal and distal stumps of transfected nerves and promoted axonal regeneration after peripheral nerve injury [49]. Recently, Alge et al. [50] evaluated the effects of Gal-1 on the migratory capacity of human retinal pigment epithelial cells. Interestingly, a significant reduction of the migratory activity was observed when Gal-1 expression was silenced by small interfering RNA (siRNA). Taken together, these results agree with cell invasiveness studies using a proteomic approach for the comparison of highly and poorly invasive mammary carcinoma cells; in these studies Gal-1 membrane expression was identified as a signature of cell invasiveness [51]. Accordingly, both the treatment with Gal-1-specific antisense oligodeoxynucleotides or polyclonal anti-Gal-1 antibodies resulted in inhibition of EC migration, which suggests an essential role for Gal-1 during angiogenesis [52], as originally proposed by pioneering experiments performed by Clausse et al. [53].

Notably, dendritic cells (DCs) derived from human monocytes showed enhanced migration across ECM when matured in the presence of Gal-1 [54]. When migration of Gal-1-treated or LPS-treated DCs was analyzed by Matrigel, Gal-1-treated DCs migrated significantly better toward an MIP-3β gradient than LPS-treated cells. In contrast, transendothelial migration across EC monolayers was similar for both cells. Consistent with the high migratory phenotype, Gal-1 induced high expression of MMP-1, MMP-10, and MMP-12 in treated DCs. Therefore, Gal-1 may serve to regulate the migratory phenotype of DC through inflammatory tissues and the ability of mature DC to respond to chemotactic signals.

Nevertheless, antagonistic effects on cell migration were also documented for Gal-1 by several authors. For instance, we have demonstrated that Gal-1 has an inhibitory effect on leukocyte migration in an *in vivo* model of acute inflammation, when the lectin was co-injected or injected before phospholipase A$_2$. This effect was almost completely abrogated by the anti-Gal-1 antibody. Histopathological studies showed a clear reduction of the inflammatory process evi-

Table 2. Cell migration and motility mediated by galectins.

Galectin type	Cell type	Migration/Motility	Ref.
Gal-1	U87 human glioblastoma	↑	45, 46
	rat hepatic stellate cells	↑	48, 102
	rat Schwann cells	↑	49
	human retinal pigment epithelial cells	↑	50
	human umbilical vein endothelial cells	↑	52
	human dendritic cells	↑	54
	rat neutrophils	↓	55
	human neutrophils	↓	56
	human eosinophils	↓	57
	human colon cancer	↓	58
	BW5147, Pha^{R}2.1, BWC2GnT murine T-cell lines	↓	60
Gal-3	human monocytes	↑	93
	human monocytes and macrophages	↑ chemoattraction	94
	human tumor astrocytes	↑	96
	human breast carcinoma	↑	73
	DLKP lung squamous cell carcinoma	↑	69
	murine thymocytes	↑ chemoattraction	99
	human colon cancer	↓	58
	U373 human glioblastoma	↓	103
Gal-8	CHO-P hamster ovarian	↑	125
	T98G, U373 human glioblastoma	↑	96
	HCT-15, CoLo201 human colon cancer	↓	135
Gal-9	human eosinophils	↑ chemoattraction	145–148
	human Jurkat T-cells	↑ chemoattraction	149

denced by a diminished number of infiltrated poly-morphonuclear neutrophils (PMNs) and a reduction in the number of degranulated infiltrating mastocytes [55]. In further studies, La et al. [56] showed that Gal-1 inhibits IL-8-induced PMN chemotaxis in a concen-tration-dependent manner. Transendothelial migra-tion assays revealed that Gal-1 inhibits IL-8-stimu-lated PMN migration through monolayers of ECs. Intravital microscopy studies in mouse mesenteric microvasculature demonstrated a critical role for Gal-1 in selective attenuation of leukocyte rolling, adhe-sion, and migration. Gal-1 has also been shown to markedly reduce the migration of eosinophils in comparison to P-selectin in *ex vivo* cultures of human nasal polyps. In summary, Gal-1 induced a decrease both in the relative distance covered by eosinophils and in their migration speed as visualized by quantitative videomicroscopy. Budesonide mark-edly increased Gal-1 expression and inhibited eosino-phil migration. These results provided another cellular target for the anti-inflammatory effects of this protein [57]. Similarly, Hittelet et al. [58] demonstrated that Gal-1 markedly decreased the motility of HCT-15, LoVo, and CoLo201 human colon cancer cells, an effect that was partially neutralized by anti-Gal-1 antibodies.

Other studies showed that pharmacological treatment with Gal-1 inhibits leukocyte recruitment into the peritoneum in a model of rat peritonitis [59]. Recent-ly, Gal-1 was shown to inhibit T-cell migration across

ECs; these cells were induced to express high levels of Gal-1 upon exposure to prostate cancer cell-condi-tioned medium. The reduction in T-cell trafficking across the EC monolayer in the presence of increased cell surface Gal-1 occurred in absence of T-cell death, and was not caused by any increase in T-cell adhesion. Similarly, when Gal-1 was added to the upper surface of Matrigel in transwell chambers, the lectin caused a reduction in T-cell migration through Matrigel which was abrogated by anti-Gal-1 antiserum. The ability of Gal-1 to inhibit T-cell migration across the endothe-lium and sites of inflammation endows Gal-1 with novel anti-inflammatory properties [60].

Altogether, these reports support the concept that Gal-1 promotes cell migration, a function that corre-lates with the ability of this galectin to influence tumor progression, angiogenesis, liver and axonal regener-ation, and other biological events. Nevertheless, it seems apparent that the biological roles of Gal-1 are tissue-specific since it also decreases cell migration of most immune cells investigated, providing a rational basis for its anti-inflammatory properties.

Galectin-2

Galectin-2 in cell-substrate adhesion

Cloning and expression of Gal-2 was first performed by Barondes' group [61, 62]. Gal-2 has been shown to exhibit a distinct expression profile, compared to

other members of the galectin family, and is mainly confined to the gastrointestinal tract [63, 64]. Rat and human Gal-2 have been pointed out as cell adhesion modulators. Gal-2 has been shown to bind to carbohydrate residues on T-cell surface proteins such as β_1 integrins, but not CD3 or CD7.

Cell adhesion studies of T-cells were performed with collagen type I or FN as matrix compounds to test if bivalent Gal-2 might bridge glycan chains of the matrix and cell surface glycoproteins. After Gal-2 treatment, adhesion was significantly reduced or enhanced when T-cells bound to collagen I or FN, respectively. Lamina propria-derived T-cells reacted in a similar manner. In contrast, Gal-1 reduced the adhesion to both matrix compounds, and Gal-7 was a rather weak effector. When T-cells were pre-incubated with integrin-specific mAbs before adding Gal-2, cell adhesion to collagen I was significantly inhibited by mAbs against β_1-, α_1-, and α_2 integrins, whereas the adhesion to FN was blocked by β_1-, α_3-, α_4-, and α_5-specific mAbs. In brief, the presence of Gal-2 on the T-cell surface enhanced adhesion to FN, and β_1-integrin was one of the main T-cell surface ligands involved, whereas Gal-1 reduced this binding under similar conditions [64]. Furthermore, it is noteworthy that some tubulin isoforms have been shown to be ligands for Gal-2 [65]. This observation might be related to regulation of microtubule polymerization or reorganization. However, the mechanisms and physiological relevance of this interaction remain to be elucidated. Further studies on Gal-2 are needed to address its putative role in cell to substrate adhesion. To date, no experimental evidence has been reported on cell-cell interactions or cell migration mediated by this lectin.

Galectin-3

Galectin-3 in cell-substrate adhesion

Positive regulation of cellular adhesion mediated by Gal-3 has been demonstrated in certain models. Exogenously added Gal-3 showed pro-adhesive properties, promoting human PMN adhesion to LN-coated plastic in a carbohydrate-dependent manner (Fig. 1 a). Truncated Gal-3, which lacks the amino-terminal region with the R domain but contains the CRD, did not promote PMN adhesion [66]. This result was explained by the fact that Gal-3 can function bivalently, and to self-associate it requires its R domain, which forms intermolecular aggregates in a concentration-dependent manner (up to 1 μM) [67, 68].

In addition, Gal-3 has been demonstrated to be essential for rapid adhesion of Gal-3-transfected versus Gal-3-null expressing BT-549 human breast carcinoma cells to LN and collagen IV, but not to FN

[69]. Interestingly, Gal-3-transfected cells showed higher levels of $\alpha_6\beta_1$ integrin [69]. Other studies demonstrated that fast spreading and adhesion of breast carcinoma cells to tissue culture plates was attained when intracellular Gal-3 was released rapidly by fetuin. Moreover, adhesion to elastin – a specific ligand of Gal-3 – was dramatically improved by Gal-3, suggesting that the lectin released by fetuin might be directly used to ligate breast carcinoma cells to elastin-rich tissues such as the lungs [70].

Likewise, overexpression of Gal-3 in Evsa-T human breast cancer cells has been shown to specifically influence cell adhesion to various plastic-immobilized ECM proteins. In fact, the adhesion of Gal-3-transfected cells to LN-, FN-, or vitronectin-coated wells was 48, 60, and 39% higher than that found for non-transfected cells, and lactose blocked the effects (Fig. 1 d, upper panel). Moreover, extensive spreading on LN-coated dishes and morphological spreading-associated features (protrusions, F-actin-positive ruffles, leading edges, and F-actin reorganization) were detected in cells overexpressing Gal-3 [22]. Similarly, normal fibroblasts engineered to overexpress Gal-3 have also been shown to reorganize the actin microfilaments in order to spread [71].

Inufusa et al. [72] have reported that anti-Gal-3 antibodies and the competitive disaccharide lactose inhibited cell adhesion to LN of the highly metastatic adenocarcinoma XK4-A3 *in vitro,* as well as liver metastasis *in vivo.* The cellular response to extracellular Gal-3 might be different depending on the tumor cell stage or cell type: i.e. primary- and late-stage breast carcinoma cells responded differently to exogenously added Gal-3, since only the former showed increased adhesiveness in Matrigel assays [73].

Complete absence of effects and negative modulation of cell adhesion to ECM proteins by Gal-3 has also been well documented. For instance, SCM-153 human breast cancer cells were shown to express Gal-3 and to adhere to LN, but the addition of lactose or anti-Gal-3 mAb failed to inhibit adhesion, suggesting that Gal-3 in these cells might not be functioning in cell adhesion [74]. Likewise, A2058 and A375 melanoma cells have been shown to express Gal-3 on their surfaces and to attach to LN *in vitro*, but Gal-3 did not affect adhesion to LN, and anti-Gal-3 antiserum did not alter cell adhesion [29]. On the contrary, other studies have shown that soluble Gal-3 blocked the adhesion and spreading of baby hamster kidney epithelial cells on LN-1-coated plates [75], as expected for matricellular proteins (Fig. 1 c).

Ochieng et al. [76] demonstrated that high levels of Gal-3 on the cell surface downregulated cellular adhesion to ECM proteins, in agreement with the cell de-adhesion hypothesis. To address this issue,

different cell types were incubated without or with rGal-3 prior to plating onto LN-, collagen IV-, or FN-coated wells. As expected, soluble Gal-3 inhibited cell adhesion to the ECM proteins (Fig. 1 c) in a dose-dependent manner with a marked inhibition at a lectin concentration of ~3 μM; this effect was abrogated in the presence of lactose.

Remarkably, Gal-3 was later demonstrated to be endocytosed and to be involved in the endocytosis of β_1 integrins (CD29) from the cell surface to intracellular vesicles via the caveolae pathway. In fact, saccharide-dependent binding of Gal-3 to the cell surface was sufficient to trigger its own endocytosis and that of CD29: preincubation of breast carcinoma cells with high concentrations of rGal-3 (~5 μM) mediated total sequestration of integrins in intracellular vesicles [77]. Similarly, Gal-3 regulation of $\alpha_4\beta_7$ integrin expression has also been reported [22]. Based on these data, a model was postulated in which moderate extracellular concentrations of Gal-3 *in vivo* are sufficient for trafficking and/or redistribution of integrins on the cell surface, thereby enhancing cellular spreading and motility, and remodeling of adhesion plaques. However, at high concentrations of Gal-3, β_1 integrins (CD29) are endocytosed by the cells and consequently remain not available for firm adhesion [77].

In conclusion, in most of the cases studied, both exogenously added recombinant Gal-3 and Gal-3 from transfected cells showed positive regulation of cellular adhesion to ECM glycoproteins. However, some reports suggest that this galectin can negatively regulate cell adhesion. These differences may probably rely on: a) galectin ligands involved in cell to substrate adhesion, b) cell-membrane receptors in each cell type for FN or LN, and c) co-expression of various galectins in the same cell type.

Galectin-3 in cell-cell adhesion

Homotypic cell adhesion mediated by Gal-3 was originally reported by Inohara and Raz [78], who showed that transfected sf9 insect cells expressing Gal-3 underwent homotypic cell adhesion in the presence of asialofetuin. Metastatic cancer cells have been demonstrated to aggregate homotypically in a Gal-3-dependent manner to form multicellular intravascular clumps at sites of primary adhesion of tumor cells to the endothelium [79]. This spontaneous carcinoma cell homotypic aggregation was shown to be, at least partly, mediated by interaction between the cell surface Thomsen-Friedenreich glycoantigen (TFAg) and Gal-3 [42, 80].

In another model, Gal-3-mediated homotypic aggregation was demonstrated to be associated with the glycoprotein 90K/Mac-2BP. This protein was shown to mediate homotypic cell adhesion by cross-linking Gal-1/-3 on adjacent cells. Both 90K/Mac-2BP and galectins were found to be deposited in the ECM, where they may interact with different matrix components, thereby mediating cell adhesion [81].

Regarding cell adhesion to the endothelium, Gal-3 has been shown to mediate MDA-MB-435 breast carcinoma cell adhesion to human EC. In fact, Gal-3 on the EC was demonstrated to interact with TFAg on MDA-MB-435 cells, and a synthetic TFAg-specific peptide, developed by phage display, inhibited this interaction as well as breast cancer cell homotypic aggregation [42]. Recently, the finding by phage display technology of two specific peptides that bind to Gal-3 CRD opened a revolutionary way to develop galectin inhibitors. These Gal-3 inhibitory peptides interfered with rolling and stable adhesion of human breast carcinoma cells to bone marrow EC, and dramatically reduced homotypic cell aggregation [82]. In fact, the modified citrus pectin – a carbohydrate ligand for Gal-3 – has been demonstrated to sequester Gal-3 and to inhibit homotypic tumor cell aggregation, tumor growth, and metastasis [83, 84].

Other studies also supported the observation that Gal-3 expressed on EC could interact with carbohydrate ligands displayed on carcinoma cells to mediate tumor cell adhesion and metastasis. For instance, PC-3 human prostate carcinoma cells, which do not express Gal-3, bind to EC in a manner that can be inhibited by anti-Gal-3 mAbs [85]. Similarly, an anti-Gal-3 mAb inhibited MDA-MB-435 human breast carcinoma cell rolling and adhesion to human umbilical vein endothelial cells (HUVECs) under flow conditions [86]. Likewise, Krishnan et al. [87] demonstrated that Gal-3 plays a key role in the adhesion of B16-F10 murine melanoma cells to the lungs. In addition, the lectin constitutively expressed on lung vascular EC surfaces served as an anchor for circulating B16-F10 cells. Alternatively, Gil et al. [88] studied the immunohistochemical localization of Gal-3 during *in vitro* human PMN adhesion and transmigration through EC monolayers and found a markedly enhanced Gal-3 expression in the plasma membrane of EC after PMN adhesion, suggesting that EC-derived Gal-3 could regulate PMN-EC interactions.

In addition, Gal-3 has been postulated as a substrate for matrix metalloproteinases such as MMP-2 and MMP-9, which are capable of efficiently cleaving the Ala^{62}-Tyr^{63} bond in the R domain of full-length Gal-3 (30 kDa), generating a 22-kDa fragment with an intact CRD [89]. MMP-2-cleaved Gal-3 displayed ~20 times greater affinity for HUVECs compared to the full-length protein [90], which might play an important role in tumor invasion and angiogenesis: i.e. cleaved Gal-3 cannot dimerize, but retains its ability to bind to

glycoconjugates, thus competing with intact Gal-3 [91].

In DCs, Gal-3 is constitutively expressed on the cell surface and is involved in the adhesion of L-selectin-activated lymphocytes. In fact, adhesion of L-selectin-triggered lymphocytes to DCs was significantly reduced by anti-Gal-3 mAbs and by specific carbohydrate ligands. High concentrations of rGal-3 inhibited the interactions between DCs and lymphocytes, an effect that was reverted by specific carbohydrate ligands. These findings strongly suggested that a Gal-3 ligand was induced on L-selectin-triggered lymphocytes to interact with Gal-3 on the cell surface of DCs [92].

In conclusion, the role of Gal-3 in modulating intercellular adhesion has been extensively documented. It appears that cell-cell adhesion is promoted in cells expressing Gal-3, and this process can be inhibited by anti-Gal-3 antibodies, or by soluble rGal-3 – as would be expected for matricellular proteins.

Galectin-3 in cell migration

Potential roles of Gal-3 in cell migration and invasiveness have been well documented in different models. Liu and colleagues described a novel role for this glycan-binding protein as a potent chemoattractant for monocytes and macrophages [93]. The authors showed that Gal-3 induced human monocyte migration both *in vitro* and *in vivo* in a dose-dependent manner. This lectin was chemotactic at concentrations >1 μM (relatively high concentrations, probably required for Gal-3 dimerization/oligomerization); this effect was specifically inhibited by an anti-Gal-3 mAb or specific carbohydrate ligands. Both the N-terminal and C-terminal domains were involved in this activity, which was mediated, at least in part, through a pertussis toxin-sensitive (G-protein-coupled) pathway. In addition, Gal-3 was shown to attract mature macrophages, which was a noteworthy finding, because, unlike monocytes, there are very few chemokines reported to be chemoattractants for differentiated macrophages (including MCP-1 – the major monocyte chemoattractant) [94]. Therefore, it is possible to speculate that Gal-3 is one of the major factors involved in the influx of macrophages to inflammatory sites. Gal-3-deficient mice consistently developed significantly reduced numbers of peritoneal macrophages compared to wild-type (WT) mice when treated with thioglycolate [95].

In addition, Gal-3 was able to markedly stimulate human tumor astrocyte migration *in vitro* in Hs683, T98G, and U373 cells cultured on Gal-3-coated plates and monitored by video cell tracking, while Gal-1 and Gal-8 were weaker stimulators. Furthermore, the three galectins appeared to be involved in tumor astrocyte invasion of the surrounding brain parenchyma, since their expression levels were higher in the most invasive parts of the xenografted glioblastomas [96].

Fittingly, Gal-3 – exogenously added to cell cultures or bound to the substrate- markedly enhanced the migration of primary breast carcinoma cells [73]. Gal-3 overexpression was associated with increased cell surface expression of the $\alpha_4\beta_7$ integrin, causing enhanced adhesion to ECM glycoproteins as well as an increase in invasiveness and spreading-associated features [22]. Similarly, Gal-3-transfected human breast carcinoma cells were shown to invade through Matrigel-coated filters at ~3 times the rate of parental cells [69]. Gal-3-transfected DLKP lung squamous cell carcinoma cells were also rendered more motile and invasive through ECM *in vitro* [97]. With respect to the migration of ECs, Gal-3 was shown to be an endothelial ligand for NG2 chondroitin sulfate proteoglycan in MAEC cells, and anti-Gal-3 mAbs inhibited NG2-induced motility by 70% [98]. In the thymus, Gal-1, -3, and -9 were found to be produced by the stroma, and Gal-3 has been reported as a modulator of thymocyte migration by interfering with cell adhesion and promoting subsequent de-adhesion. Gal-3 purified from thymic epithelial cells, alone or in combination with LN, displayed chemotactic effects, especially on $CD4^+ CD8^+$ thymocytes, although this lectin significantly inhibited adhesion of thymocytes to epithelial cells [99].

In very elegant studies, overexpression of the enzyme β1,6 N-acetylglucosaminyltransferase V (Mgat5), which catalyzes the addition of β1,6GlcNAc to N-glycans leading to subsequent elongation with poly-LacNAc, has been shown to increase cell motility and tumor formation, while a Mgat5-deficient phenotype suppressed mammary tumor growth and metastasis in polyoma middle T transgenic mice [100]. Addition of low concentrations of rGal-3 (up to 2 μg/ml) to the culture medium stimulated FN fibrillogenesis and FN-dependent cell spreading and motility in $Mgat5^{+/+}$ tumor cells plated on an FN substrate, in a carbohydrate-dependent manner. Gal-3 stimulated integrin-mediated activation of focal adhesion kinase (FAK) and phosphatidylinositol 3-kinase (PI3K) as well as $\alpha_5\beta_1$ translocation to fibrillar adhesions [101]. On the contrary, Gal-3 did not alter the motility of rat hepatic stellate cells, although Gal-1 significantly augmented their migratory phenotype [102]. Notably, Hittelet and colleagues [58] demonstrated that addition of Gal-3 on Matrigel-coated substrate strongly decreased the motility of HCT-15 and, to a lesser extent, of LoVo and CoLo201 human colon cancer cells; this effect could not be neutralized by anti-Gal-3 antibodies. In human glioblastoma U373 cells, down-

regulation of Gal-3 using antisense strategies generated knockdown cells with significantly higher motility when cultured on LN-coated substrates as compared to non-transfected control cells. Likewise, knocking down Gal-3 expression in glioma cells resulted in increased glioma cell motility on LN substrates [103].

The above-mentioned evidence strongly supports the concept that Gal-3 regulates cell migration. It promotes monocyte migration, and it is considered as one of the major factors mediating the influx of macrophages to sites of inflammation. In addition, Gal-3 positively regulates the motility of various tumor cells, whereas it reduces the migration of colon and glioblastoma cancer cell lines. These opposing results might be related to differences among target cells that may include different cell surface receptors for Gal-3 and for FN/LN, as well as distinct expression profiles of multiple galectins present in each cell type.

A better understanding of the role of Gal-3 in cell-matrix interactions, cell-cell adhesion, and cell migration will be critical to examine the role of this endogenous lectin as a candidate target for therapeutic intervention. It is important to keep in mind that cell surface glycoconjugates are tissue-specific, and can differ significantly among cell types. Moreover, Gal-3 can modulate the expression, cell surface distribution, and endocytosis of some receptors (e.g. integrins, which play an important role in cell adhesion and migration).

Galectin-4

Galectin-4 in cell adhesion

Gal-4 was initially cloned from rat intestine [104], and its localization was found to be restricted to the epithelium of the alimentary tract, including oral mucosa, esophagus, and intestinal mucosa [105–108]. Similarly to other galectins, Gal-4 has been implicated in cell adhesion processes. In fact, plate-coated Gal-4 supported adhesion of the T84 human carcinoma cell, and this effect was significantly inhibited by lactose in a dose-dependent manner, implying that the immobilized lectin interacted with one or more receptors at the cell surface in a carbohydrate-dependent manner. Therefore, Gal-4 might play a role in the initial attachment and/or spreading of cells during cell adhesion or cell migration. This effect might have potential implications in complex physiological processes such as restitution of intestinal epithelium, maintenance of epithelial integrity, and epithelial wound healing [109]. Moreover, Gal-4 was localized by immunohistochemistry at the leading edge in lamellipodia of subconfluent human colon adenocarcinoma T84 cells, and in attachment sites of newly seeded cells, confirming its role in cell-substrate adhesion.

In addition, Chiu et al. [105, 106] found the presence of Gal-4 in adherens junctions of porcine tongue squamous epithelium and in globular structures at the cell periphery. On the bases of its colocalization with actin, vinculin, and uvomorulin, this lectin was suggested to be an adherens junction protein in oral epithelial cells. However, other roles for Gal-4 such as stabilization of cellular junctions and membranes have been proposed: Gal-4 localization in the apical surface of the enterocytes was associated to its potential function as a central organizer/stabilizer of lipid rafts in the microvillar membrane [110], rather than to a participation in local cell-cell or cell-matrix interactions. In fact, pig small intestinal Gal-4 was localized at the brush border membrane in enterocytes, and it was considered as an intestinal brush border protein associated with its potential natural ligands, such as aminopeptidase N and sucrase-isomaltase (major digestive enzymes confined to the apical enterocyte surface) [107]. Finally, Wasano and Hirakawa [111] also proposed a role for Gal-4 in stabilizing the apical (lumenal) membranes in rat esophageal epithelium. Further studies are required to dissect the precise roles of Gal-4 in the regulation of cell adhesion and migration under physiological and pathological circumstances.

Galectin-7

Galectin-7 in cell adhesion and migration

Gal-7 was almost simultaneously cloned from human keratinocytes by Madsen et al. [112] and Magnaldo et al. [113]. Gal-7 is the first marker of epithelial stratification whose expression does not depend on local differentiation; this lectin is present in all subtypes of keratinocytes, in all cell layers in epidermis, and also in the cornea and esophagus. Gal-7 is secreted by proliferating, quiescent, as well as differentiated keratinocytes into the culture medium [114]. This protein, which has been designated as a product of the *p53-induced gene 1* (PIG1), was and is among the most highly induced genes in p53-transfected DLD-1 colon carcinoma cells [115], suggesting its potential role in the regulation of apoptosis and cell cycle progression [116].

Gal-7 is localized at sites of cell-to-cell contact, particularly in the upper layers of the human epidermis [112], but *in vitro* adhesion assays have not been performed. Therefore, its putative role in adhesion is speculative. When transfected into lymphoma cells, Gal-7 was able to promote *MMP-9* gene expression

[117], suggesting its pivotal role in the regulation of migration and dissemination of tumoral cells, at least in those cancers arising from pluristratified epithelia (i.e. some types of head and neck squamous cell carcinomas) [114].

Potential effects of Gal-7 on cell migration and spreading have been suggested by other authors. In HeLa cells engineered to overexpress Gal-7, four- to seven-fold increases were detected by microarray analysis in mRNA levels for α_1 integrin and for ECM proteins such as vitronectin and type XV collagen, respectively [116]. Thus the possibility still remains that Gal-7 might be interacting with integrins to mediate cell migration.

Gal-7 has also been implicated as a potential mediator of epithelial cell migration in re-epithelization of corneal wounds [118, 119]; healing corneas contained significantly increased levels of Gal-7 compared with normal corneas, and wound closure was significantly stimulated by Gal-7, but not by Gal-1. Moreover, immunohistochemical studies in healing corneas showed strong Gal-7 staining in the leading edge of the migrating epithelium, in the peripheral epithelium, and at sites of cell-matrix interactions [119]. Further *in vitro* and *in vivo* studies are needed to evaluate the direct involvement of Gal-7 in cell adhesion and migration.

Galectin-8

Galectin-8 in cell-substrate adhesion

Gal-8 is a bi-CRD galectin originally cloned from a rat liver cDNA library by Zick's group [120]. Gal-8 is highly expressed in invasive human prostate carcinoma where it was first identified as *prostate carcinoma tumor antigen-1* (PCTA-1) [121]. Alternative splicing of *gal-8* mRNA generates different transcripts encoding, at least, six different protein isoforms of human Gal-8 that are tissue-specific [122]. Gal-8 seems to be unique in the galectin family, with isoforms belonging to both the prototype and tandem-repeat groups. At least two splice variants of human Gal-8 are of the tandem-repeat type, and differ in the length of the linker peptide or hinge region. One of the tandem-repeat wild-type isoforms, designated Gal-8M (*Gal-8 medium*), possesses a medium-sized linker peptide without a thrombin cleavage site, while the other bi-CRD isoform, called Gal-8L (*Gal-8 long*), contains the longest linker peptide that includes a thrombin recognition domain [123, 124]. It has been proposed that the linker peptide provides not only cross-linking ability without the formation of a dimer/multimer structure, but also confers protease susceptibility.

Very elegant cell adhesion studies revealed that Gal-8 can positively or negatively regulate cell adhesion, depending on the extracellular microenvironment. When immobilized onto matrix, Gal-8 can be classified as a novel ECM protein with a similar potency to FN in promoting cell adhesion and spreading due to ligation of sugar moieties present in cell surface integrin receptors. Actually, cells adhered and spread onto plates coated with Gal-8 with a kinetics similar to that found when cells attached to FN [125].

In fact, the mechanism of interaction of Gal-8 with integrins involves lectin binding to sugar moieties (protein-glycan interactions), whereas the ligand-binding site on the extracellular domain of the integrin molecules interacts with FN (protein-protein interactions). Moreover, Gal-8 probably induced aggregation of β_1 integrin subunits, which are the main upstream activators of FAK (focal adhesion kinase) [125]. In this sense, Gal-8 resembles the classical ECM proteins that induce integrin aggregation to trigger cell adhesion and to initiate integrin-mediated signaling cascades [126]. Immobilized truncated Gal-8 containing only one CRD corresponding to the N-half of the protein, termed N-Gal-8, was ~5-fold less potent than wild-type Gal-8 in promoting cell adhesion, suggesting that occupancy of both Gal-8 CRDs is required to modulate cellular adhesion [125].

Soluble Gal-8 has been shown to selectively inhibit cell adhesion to plates coated with LN and FN (Fig. 1c), while it fails to inhibit cell adhesion to the non-specific substrate polylysine. In this regard, Gal-8 also resembles other soluble ECM proteins like LN [127] and FN [128] in that a cell adhesion protein might become a specific inhibitor of its own function if it is bound in excess to a cell receptor. Hence, saturation of these cell receptors might prevent receptor interactions with substrate- or cell attached-adhesive molecules. In fact, the addition of excess soluble Gal-8 presumably masks integrin binding sites and thus impairs cell adhesion to integrin ligands such as FN.

In line with this idea are the experiments demonstrating inhibition of colony formation in 1299 human lung carcinoma cells transfected with the *gal-8* gene: the inhibitory effect of overexpressed Gal-8 (Fig. 1d, lower panel) could account for an autocrine effect of the secreted lectin that interacted with the available cell surface integrins, similar to the inhibitory effect of Gal-8 when exogenously added to cells [129]. In addition, as mentioned above for Gal-3, soluble Gal-8 could alternatively induce the internalization of cell surface integrins, in such a way impairing cell adhesion [77].

Altogether, the anti-adhesive effects of Gal-8 could be mediated either upon direct binding of excess soluble

Gal-8 to cell surface integrins, or upon binding of ECM proteins such as FN that, when soluble, could exert an anti-adhesive effect of their own – i.e. soluble calf serum FN can bind Gal-8. Because of their anti-adhesive functions, Gal-8 (Fig. 1 c), as well as Gal-1 and Gal-3 may be considered as novel members of the adhesion-modulating proteins collectively known as matricellular proteins, which include SPARC, thrombospondin, tenascin, hevin, and disintegrins [19]. These proteins do not serve as integral components of matrix elements, but rather function through binding to matrix proteins as well as to cell surface receptors [125].

The assumption that integrins are indeed the key mediators of the inhibitory effects of Gal-8 on cell adhesion is based on evidence of the direct binding of Gal-8 to α_3, α_6, and β_1 and, to a very limited extent, to α_4 and β_3 integrins in HeLa and 1299 cells [129]. It has been hypothesized that Gal-8 acts as an integrin binding-protein that exerts down-modulatory effects on integrin functions (i.e. by a mechanism involving phosphorylation of the β_1 integrin cytoplasmic domain), instead of generating steric hindrance by interacting primarily with cell recognition sites for integrins or other adhesion receptors on ECM proteins [129]. Different galectins might selectively regulate interactions of integrins with matrix proteins in a somewhat different fashion: for example, Gal-1 might interact with integrins mainly interfering with LN-integrin interactions (i.e. $\alpha_7\beta_1$ [33]).

During cell adhesion onto immobilized Gal-8, phosphorylation of downstream effectors of PI3K, such as protein kinase B (PKB), $p70^{S6}$ kinase ($p70^{S6}$K), and extracellular-regulated kinase (ERK)-1 and -2, was significantly higher compared to adhesion to immobilized FN [130]. Zick and colleagues proposed that Gal-8 can act in three different modes, depending on the cellular context and the extracellular environment. When present at low concentrations as an immobilized ligand (even in the presence of serum or selected growth factors), this lectin interacts only with high-affinity receptors of the integrin family that promote cell adhesion, spreading, and cell migration (Fig. 1 b). In contrast, when Gal-8 is present at high enough concentrations as a soluble ligand or when it is overexpressed and secreted, it can interact with low-affinity receptors (other members of the integrin family or different cell surface receptors) that trigger signaling pathways involving the activation of stress-activated kinases like JNK and the expression of the cyclin-dependent kinase inhibitor p21. The accumulation of p21 induced by soluble Gal-8 protects the cells from potential pro-apoptotic signals and produces cytostatic effects. The third mode of action (the pro-apoptotic effect) is exhibited either under condi-

tions that prevent the accumulation of p21 or following a sustained deprivation of growth factors [131].

Proper functioning of Gal-8 depends not only upon the presence of its two CRDs but also upon their orientation, determined by the length of the hinge (linker) region. Deletion of the hinge region or single mutations in some of the residues involved in sugar binding (W^{85} and W^{248}) severely impaired the adhesive/anti-adhesive and signaling capacities of Gal-8. Truncated Gal-8 containing only the CDR corresponding to the N-half of the protein, although capable of sugar binding, was less potent than Gal-8 in promoting cell adhesion and spreading onto itself, and completely ineffective when added soluble in inhibiting cell adhesion to FN-coated plates. A Gal-8 truncated form containing only the C-CDR was devoid of any adhesive/anti-adhesive activity. In summary, Gal-8 probably requires cooperative interactions between the two CRDs and a properly oriented hinge region for effective function [132].

Gal-8 was found to induce firm adhesion of peripheral blood PMN, which was blocked by lactose [133]. Tissue culture plates precoated with a serum-free Gal-8 (Fig. 1 b) (0.3–10 μM) solution supported PMN adhesion; however, the levels of adhesion were lower than 40% of those induced by soluble Gal-8 (1 μM) when PMN adhered to untreated culture plates. The integrin α_M and the promatrix metalloproteinase-9 (proMMP-9) were identified as Gal-8 ligands, and anti-α_M mAbs – but not α_L – strongly inhibited PMN adhesion induced by Gal-8. Abolition of the sugar-binding activity of C-terminal CRD, but not N-terminal CRD, abolished the adhesion-inducing activity of Gal-8. Thus, Gal-8 appears to be a new player in PMN migration and a modulator of MMP-9 activity, necessary for matrix degradation [133]. Furthermore, a mutant form of Gal-8 lacking the entire linker region (Gal-8-null) allowed the authors to demonstrate that removal of the linker peptide greatly increased the protease resistance of Gal-8 to elastase and trypsin. Gal-8-null induced PMN adhesion in a manner comparable to that of Gal-8M (an isoform of Gal-8 with a medium-sized linker peptide), suggesting that removal of the entire hinge greatly improved stability against proteolysis without negative effects on PMN adhesion [123]. Moreover, thrombin treatment of the isoform Gal-8L (an isoform of Gal-8 with a long-sized linker peptide) significantly suppressed its adhesion-inducing activity by 80% [124, 133].

In order to study the adhesive properties of Gal-8 on T-cells, human Jurkat T-cells were plated onto immobilized recombinant Gal-8, glutathione-S-transferase (GST)-Gal-8, or FN. In fact, similar adhesion rates were obtained for each matrix, and as expected, TDG significantly inhibited attachment to Gal-8 and GST-

Gal-8 (Fig. 1 b). Moreover, extensive spreading was also observed when Jurkat T-cells were plated onto Gal-8. This effect was accompanied by a polarized phenotype, and PI3K-dependent ERK-1, -2 activation. Integrins α_1, α_3, α_5, and β_1 were found to be Gal-8 ligands in Jurkat cells, since anti-α_5 and -β_1 blocking mAbs inhibited ~60% adhesion onto immobilized Gal-8. Interestingly, anti-Gal-8 autoantibodies from patients with systemic lupus erythematosus blocked the adhesion of Jurkat cells onto Gal-8-coated plates by more than 80% [134]. Remarkably, no experimental data have been reported to date on cell-cell interactions mediated by Gal-8.

Collectively, these data indicate that Gal-8 can either promote or inhibit cell- substrate adhesion, depending on whether it is presented as an immobilized or soluble ligand, sharing typical features of matricellular proteins. These distinct modes of action depend on the lectin concentrations, the cellular context, and the extracellular environment.

Galectin-8 in cell migration

Immobilized Gal-8 has been demonstrated to be equipotent to FN in supporting cellular migration when added to culture medium in the presence of serum. In fact, CHO-P cells seeded in agarose doplets readily sprouted and migrated on plates coated both with FN and Gal-8 following 6–8 days of growth [125]. This immobilized lectin also significantly stimulated glioblastoma cell migration *in vitro*. This observation was derived from experiments in which human astrocytic tumor cells were grown on regular plastic supports in the absence or presence of Gal-1, Gal-3, or Gal-8, and followed up by video cell tracking. Under these experimental conditions, Gal-8 significantly increased the levels of migration of T98G and U373 cells. Remarkably, histopathological evaluation of Gal-8 expression in human astrocytic tumors revealed higher immunopositive staining in blood vessel walls compared to the rest of tumor parenchyma and stroma, while the reverse feature was observed for Gal-1 [96], suggesting that Gal-8 derived from intratumoral EC might be involved in tumor cell migration and metastasis. In contrast, when human colon cancer cells were plated onto Matrigel, Gal-8 markedly decreased migration rates in HCT-15 and CoLo201 cells, an effect partially blocked by anti-Gal-8 antibodies. However, neither Gal-8 nor anti-Gal-8 antibodies were capable of altering the migration of LoVo or DLD-1 cells on Matrigel [135]. In conclusion, when immobilized, Gal-8 can stimulate cell migration. In contrast, soluble Gal-8 can also reduce *in vitro* cell migration, an effect expected for a matricellular protein. Thus, Gal-8 represents a novel matricellular protein, but the detailed mechanism(s) of action remains to be elucidated.

Galectin-9

Galectin-9 in cell-substrate adhesion

Gal-9 was first cloned from mouse embryonic kidney [136], and further identified in human T lymphocytes as a specific eosinophil chemoattractant [so-called ecalectin (ECA)] [18]. This lectin is a bi-CRD galectin with three splicing isoforms which are named according to the length of the linker peptide: a) long-sized (Gal-9L, with 58 amino acids in the linker peptide), b) medium-sized (Gal-9M, 26 amino acids), and c) short-sized (Gal-9S, 14 amino acids) [18].

Regarding cell adhesion to substrate, Asakura et al. [137] demonstrated adhesion of human peripheral blood eosinophils to Gal-9-coated dishes (30 nM). In these experiments, significant adhesion was achieved, which was partially inhibited by lactose. In contrast, eosinophils failed to adhere to Gal-1-coated plates, indicating that Gal-9 selectively mediates eosinophil adhesion. Furthermore, this effect was cell-specific since PMN did not adhere to Gal-9-coated dishes under the same experimental conditions. Irie et al. [138] evaluated the adhesion of the MCF-7 human breast cancer cell line transfected with expression vectors for the three isoforms of Gal-9 (S, M, and L) on collagen type IV, FN, LN, and vitronectin. Results showed that cells expressing the Gal-9 S and L exhibited reduced adhesion to LN, vitronectin, FN, and collagen type-IV (Fig. 1 d, lower panel). In this regard, adhesion to FN or collagen type I of the Ca9–22 human oral squamous cell carcinoma cell line transfected with the *gal-9* gene was evaluated by Kasamatsu et al. [139]. These results showed that *gal-9*-transfected Ca9–22 cells showed significantly increased adhesion to FN- and collagen type I-coated plates compared to non-transfected control cells. Thus, although as a substrate Gal-9 promotes eosinophil adhesion, when Gal-9 is overexpressed in different human carcinoma cells, it can either enhance or reduce adhesion to ECM proteins, probably depending on the target cell studied.

Galectin-9 in cell-cell adhesion

Gal-9 was found to mediate eosinophil adhesion to the vascular endothelium. This lectin was found to be upregulated by interferon (IFN)-γ, and was implicated in the adherence of eosinophils to activated HUVECs *in vitro* [140]. EoL-1 human eosinophilic leukaemia cells (differentiated to eosinophils) showed increased binding to HUVECs treated with synthetic double-stranded RNA poly-IC in a lactose- and anti-

Gal-9-inhibitable manner [141]. Given these findings, it has been hypothesized that Gal-9 might be critically involved in the interactions between EC and leukocytes during leukocyte trafficking and locomotion. Upregulation of Gal-9 in ECs may probably favor the adhesion and recruitment of eosinophils as an important step in the initiation and perpetuation of inflammatory and allergic reactions. In this regard, endogenous Gal-9 mediated adhesion of eosinophils (but not PMN) to IFN-γ-activated HFL-1 fibroblasts, an effect which was specifically abrogated by lactose and anti-Gal-9 antibodies.

Cell aggregation by Gal-9 was studied by several authors. Eosinophil aggregation mediated by ecalectin was demonstrated by Matsumoto et al. [142], which was dose-dependent and lactose-inhibitable. In melanoma cells, exogenously added Gal-9 induced cell aggregation in a lactose-inhibitable fashion, suggesting that the interaction of Gal-9 on the surface of melanoma cells with its ligand was required for cell aggregation [143]. Similarly, aggregation of MCF-7 human breast cancer cells was observed following stable transfection with constructs for each of the three different Gal-9 isoforms (S, M, and L) [138]. Finally, recent work was also performed by Zhu et al. [144] who detected aggregation and formation of large clusters of Th1-, but not Th2 lymphocytes *in vitro* induced by Gal-9, identifying this lectin as a Tim-3-specific ligand and suggesting that Tim-3/Gal-9 interactions might have evolved to ensure Th1 cell apoptosis and to prevent prolonged inflammation.

Taken together, these results demonstrate that Gal-9 is involved in eosinophil adhesion to endothelial cells, suggesting possible roles in eosinophil recruitment and infiltration during the process of fibrosis, and in aggregation of eosinophils and malignant cells.

Galectin-9 in cell migration

Gal-9 acts as a selective chemoattractant for eosinophils. Gal-9 induced ~4-fold more eosinophils to migrate than the optimal dose of recombinant IL-5. Gal-9 did not induced chemotaxis of peripheral blood PMNs, lymphocytes, or monocytes. However, Gal-9 has potent eosinophil chemotactic activity *in vitro* and *in vivo* [145]. Chemotactic activity of this tandem-repeat lectin was found to be dependent on both CRDs [146, 147]. In fact, Gal-9 exhibited significantly high and dose-dependent chemotactic activity, whereas the N-terminal CRD and the C-terminal CRD truncated proteins or Gal-8 showed low activity (10%) at high concentrations. In contrast, Gal-1 and Gal-3 failed to exhibit detectable chemotactic activity, and did not inhibit Gal-9 function, suggesting that different receptors were recognized by each lectin. However, eosinophil ligands for Gal-9 have not yet been identified in eosinophils. Chemotactic activity was not reconstituted by the combination of the N-terminal CRD and the C-terminal CRD, and was inhibited almost completely by lactose. Site-directed mutants of the N-terminal or C-terminal CRD (in Arg[65] and Arg[239], respectively, two amino acids directly involved in carbohydrate recognition) failed to exhibit detectable chemotactic activity. In conclusion, the two CRDs need to be covalently linked for full chemotactic activity [146]. Furthermore, Gal-9L and Gal-9M isoforms exhibited comparable chemotactic activity [148]. Thrombin treatment of Gal-9, which cleaves within the linker peptide, caused loss of chemotactic function only in Gal-9L, as clearly demonstrated by Nishi et al. [124]. Jurkat T-cells constitutively expressed the isoforms of Gal-9 corresponding to the medium- and long-sized linker peptide (26 or 58 amino acids). Moreover, Gal-9 expression was upregulated by phorbol esters, which also stimulated chemotactic activity of Jurkat T-cells [149].

In summary, Gal-9 acts as a potent chemoattractant for eosinophils. The regulation and function of Gal-9 under various physiological and pathological conditions, however, remain to be elucidated.

Other galectins

To the best of our knowledge, *in vitro* assays to assess cell-substrate or cell-cell interactions have not been reported in the literature for Gal-5, -6, -10, -11, -12, -13, -14, or -15. However, emerging experimental evidence such as expression and localization patterns of certain galectins might allow some speculation regarding their extracellular role(s). For example, expression of Gal-6 in the gastrointestinal tract during embryonic development correlates with stages of major changes in cell-cell interactions in the intestinal epithelium [150]. In addition, specific localization of Gal-11 at the interface between lens fiber cells is also suggestive of a potential role for this protein in cellular adhesion [1, 3]. Furthermore, Gal-15 is highly expressed in the endometrial luminal epithelium, and it has been proposed as a heterophilic cell adhesion molecule between the conceptus trophectoderm and endometrial luminal epithelium. Therefore, this lectin is a likely candidate as a mediator of interactions between the endometrius and the conceptus during the process of implantation [151]. In fact, Gal-15 contains predicted cell attachment sequences (i.e. RGD) that could mediate binding to integrins [152]. Further studies on novel members of the galectin family should be carried out to elucidate potential roles of these glycan-binding proteins in cell adhesion or migration.

Concluding remarks

Because of their anti-adhesive as well as adhesive extracellular functions, galectins can be considered as a novel class of adhesion-modulating proteins collectively known as matricellular proteins (which include SPARC, thrombospondin, tenascin, hevin, disintegrins, etc.) as originally proposed by Zick and colleagues for Gal-8 [125]. Accordingly, galectins sometimes have de-adhesive effects when presented as soluble proteins to cells in a strong adhesive state. In this context the de-adhesive properties of galectins should be considered as physiologically relevant as the pro-adhesive effects of these glycan-binding proteins [153].

Galectins modulate cell adhesion/de-adhesion through different mechanisms. These glycan-binding proteins can specifically recognize biomatrix structural glycoproteins such as LN, FN, and vitronectin as well as cell surface integrins in a carbohydrate-dependent manner. Contradictory observations related to adhesive/anti-adhesive effects are likely to be due to several problems. First, the interpretation of results obtained for one galectin will only be unequivocal if no additional galectins or galectin ligands with overlapping or opposing functions would be expressed in the same tissue or cell. Second, a careful examination of experimental discrepancies in terms of concentrations employed is needed (i.e. high concentrations of soluble Gal-3 would promote β_1 integrins and Gal-3 endocytosis). Third, the valency/oligomerization state of each galectin is a crucial parameter for biological activities. Forth, extra- or intracellular cleavage is one possible mechanism for down-modulating the adhesive activity of Gal-3, Gal-8, and Gal-9. Fifth, the existence of tissue-specific cell surface receptors for galectins, with distinct glycosylation patterns, can clearly influence the effects of galectins. Finally, cell-surface receptors for each of ECM glycoprotein (LN, FN, etc.) are different for each cell type or tissue.

Regarding the co-expression of multiple galectins in a given cell type or tissue, controversial results on their role(s) in adhesion are probably due to overlapping/opposite functions. By RT-PCR analysis of galectin gene expression in a panel of 61 tumor cell lines, Lahm et al. [154] showed an excellent example of galectin co-expression in the Colo201 human colon cancer cell line which expresses not only Gal-1 and -3 mRNA, but also Gal-4, -7, -8, and -9 mRNA. This complex pattern clearly demonstrates that studies should consider potential functional redundancy, and interactions between individual galectin types. In fact, this review summarizes many experiments in which this point has not been taken into account. It will be essential to evaluate the combined effect of multiple galectins expressed in each cell type or tissue to appreciate their full functional spectrum in the modulation of cell adhesion and migration.

Concerning LN/FN cellular ligands, different cell types can interact differentially with FN or LN through their repertoire of integrins and via other receptors. For example, LN can interact with integrins and cell-surface sulfated glycolipids (sulfatides) [155]. Moreover, a given integrin such as $\alpha_4\beta_1$ binds FN not only by the arginine-glycine-aspartic acid (RGD) sequence of the FN cell-binding site, but also in the heparin-binding domain [156]. Consequently, the interactions between LN/FN and non-galectin cell surface receptors may also contribute to the complex discordant data.

Therapeutic applications of galectins based on their effects on cell adhesion have also been suggested. The concept of anti-adhesive galectin therapy [157] was originally proposed by early pioneering studies of Raz and colleagues [158, 159]. In this regard, two experimental approaches seem to be promissory as galectin inhibitors: short synthetic peptides and carbohydrate-based compounds. In this sense, short synthetic peptides as pharmacological agents aimed to interfere with tumor cell interactions may have special significance for the development of new anti-adhesive therapies. Phage display technology has allowed the development of: 1) a peptide specific for TFAg, a Gal-3 ligand [42], and 2) synthetic peptides specific for Gal-3 CRD [82]. These peptides exhibited binding to Gal-3 expressed on tumor cells and inhibited homotypic adhesion of human breast cancer cells as well as their heterotypic adhesion to EC. Regarding carbohydrate-based galectin inhibitors, Pienta et al. [83] reported that modified citrus pectin – a Gal-3 ligand – was an effective inhibitor of B16-F1 murine melanoma lung cell colonization as well as rat prostate cancer metastasis. Moreover, synthetic analogues of naturally occurring conjugates of carbohydrates and amino acids (glycoamines) have also been shown to generate efficient inhibition of cancer metastasis [40, 160]. Notably, tumor-immune escape allows malignant progression, and Gal-1 has a crucial role in conferring immune privilege due to its pro-apoptotic activity on activated T-cells and its ability to skew the balance toward a Th2 and T regulatory-mediated anti-inflammatory response. In a murine model of melanoma, we found that blockade of Gal-1 within the tumor tissue resulted in enhanced Th1-mediated antitumor responses and increased tumor rejection [161]. The ability of galectins to regulate the migration and invasiveness of tumor and inflammatory cells might also be targeted for therapeutic purposes. For example, knocking down Gal-1 expression, at least in

gliomas, has been shown to impair cell migration, invasiveness, and metastasis [46, 162]. Lactosylated steroids also blocked *in vitro* migration of human U373 glioblastoma, A549 non-small-cell lung and PC-3 prostate cancer cells [163]. Finally, anti-galectin compounds such as lectin-specific synthetic peptides and siRNA are being developed by different groups, opening a new era of non-toxic therapeutic strategies for the treatment of inflammatory and neoplastic diseases.

1 Cooper, D. N. W. (2002) Galectinomics: finding themes in complexity. Biochim. Biophys. Acta 1572, 209 – 231.

2 Vasta, G. R., Ahmed, H. and Odom, E. W. (2004) Structural and functional diversity of lectin repertoires in invertebrates, protochordates and ectothermic vertebrates. Curr. Op. Struct. Biol. 14, 617 – 630.

3 Ogden, A.T., Nunes, I., Ko, K., Wu, S., Hines, C. S., Wang, A.-F., Hegde, R. S. and Lang, R. A. (1998) GRIFIN, a novel lens-specific protein related to the galectin family. J. Biol. Chem. 273: 28889 – 28896.

4 Hirabayashi, J. and Kasai, K. (1993) The family of metazoan metal-independent β-galactoside-binding lectins: structure, function and molecular evolution. Glycobiology 3, 297 – 304.

5 Houzelstein, D., Goncalves, I. R., Fadden, A. J., Sidhu, S. S., Cooper, D. N. W., Drickamer, K., Leffler, H. and Poirier, F. (2004) Phylogenetic analysis of the vertebrate galectin family. Mol. Biol. Evol. 21, 1177 – 1187.

6 Elola, M. T., Chiesa, M. E., Alberti, A. F., Mordoh, J. and Fink, N. E. (2005) Galectin-1 receptors in different cell types. J. Biomed. Sci. 12, 13 – 29.

7 Nakahara, S., Oka, N. and Raz, A. (2005) On the role of galectin-3 in cancer apoptosis. Apoptosis 10, 267 – 275.

8 Liu, F.-T, Patterson, R. J. and Wang, J. L. (2002) Intracellular functions of galectins. Biochim. Biophys. Acta 1572, 263 – 273.

9 Hughes, R. C. (1999) Secretion of the galectin family of mammalian carbohydrate-binding proteins. Biochim. Biophys. Acta 1473, 172 – 185.

10 Nickel, W. (2005) Unconventional secretory routes: direct protein export across the plasma membrane of mammalian cells. Traffic 6, 607 – 614.

11 Cooper, D. N. and Barondes, S. H. (1990) Evidence for export of a muscle lectin from cytosol to extracellular matrix and for a novel secretory mechanism. J. Cell. Biol. 110, 1681 – 1691.

12 Seelenmeyer, C., Wegehingel, S., Tews, I., Künzler, M., Aebi, M. and Nickel, W. (2005) Cell surface counter receptors are essential components of the unconventional export machinery of galectin-1. J. Cell. Biol. 171, 373 – 381.

13 Mehul, B. and Hughes, R. C. (1997) Plasma membrane targeting, vesicular budding and release of galectin 3 from the cytoplasm of mammalian cells during secretion. J. Cell Sci. 110, 1169 – 1178.

14 Delacour, D., Gouyer, V., Zanetta, J. P., Drobecq, H., Leteurtre, E., Grard, G., Moreau-Hannedouche, O., Maes, E., Pons, A., Andre, S. et al. (2005) Galectin-4 and sulfatides in apical membrane trafficking in enterocyte-like cells. J. Cell. Biol. 169, 491 – 501.

15 Lukyanov, P., Furtak, V. and Ochieng, J. (2005) Galectin-3 interacts with membrane lipids and penetrates the lipid bilayer. Biochem. Biophys. Res. Commun. 338, 1031 – 1036.

16 Menon, R. P. and Hughes, R. C. (1999) Determinants in the N-terminal domains of galectin-3 for secretion by a novel pathway circumventing the endoplasmic reticulum-Golgi complex. Eur. J. Biochem. 264, 569 – 576.

17 Lipkowitz, M. S., Leal-Pinto, E., Cohen, B. E. and Abramson, R. G. (2004) Galectin-9 is the sugar-regulated urate transporter/channel UAT. Glycoconj. J. 19, 491 – 498.

18 Hirashima, M., Kashio, Y., Nishi, N., Yamauchi, A., Imaizumi, T. A., Kageshita, T., Saita, N. and Nakamura, T. (2004) Galectin-9 in physiological and pathological conditions. Glycoconj. J. 19, 593 – 600.

19 Bornstein, P. and Sage, E. G. (2002) Matricellular proteins: extracellular modulators of cell function. Curr. Opin. Cell Biol. 14, 608 – 616.

20 Murphy-Ullrich, J. E. (2001) The de-adhesive activity of matricellular proteins: is intermediate cell adhesion an adaptive state? J. Clin. Invest. 107, 785 – 790.

21 Kim, H. R., Lin, H. M., Biliran, H. and Raz, A. (1999) Cell cycle arrest and inhibition of anoikis by galectin-3 in human breast epithelial cells. Cancer Res. 59, 4148 – 4154.

22 Matarrese, P., Fusco, O., Tinari, N., Natoli, C., Liu, F.T., Semeraro, M. L., Malorni, W. and Iacobelli, S. (2000) Galectin-3 overexpression protects from apoptosis by improving cell adhesion properties. Int. J. Cancer 85, 545 – 554.

23 Allen, H. J., Sucato, D., Woynarowska, B., Gottstine, S., Sharma, A. and Bernacki, R. J. (1990) Role of galaptin in ovarian carcinoma adhesion to extracellular matrix *in vitro*. J. Cell. Biochem. 43, 43 – 57.

24 Ahmed, H., Sharma, A., DiCioccio, R. A. and Allen, H. J. (1992) Lymphoblastoid cell adhesion mediated by a dimeric and polymeric endogenous beta-galactoside-binding lectin (galaptin). J. Mol. Recognit. 5, 1 – 8.

25 Shrincosky, D. M., Allen, H. J. and Bernacki, R. J. (1993) Galaptin-mediated adhesion of human ovarian carcinoma A121 cells and detection of cellular galaptin-binding glycoproteins. Cancer Res. 53, 2667 – 2675.

26 Ramkumar, R. and Podder, S. K. (2000) Elucidation of the mechanism of interaction of sheep spleen galectin-1 with splenocytes and its role in cell-matrix adhesion. J. Mol. Recognit. 13, 299 – 308.

27 Zhou, Q. and Cummings, R. D. (1993) L-14 lectin recognition of laminin and its promotion of *in vitro* cell adhesion. Arch. Biochem. Biophys. 300, 6 – 17.

28 Mahanthappa, N. K., Cooper, D. N. W., Barondes, S. H. and Schwarting, G. A. (1994) Rat olfactory neurons can utilize the endogenous lectin, L-14, in a novel adhesion mechanism. Development 120, 1373 – 1384.

29 van den Brule, F. A., Buicu, C., Baldet, M., Sobel, M.E., Cooper, D. N., Marschal, P. and Castronovo, V. (1995) Galectin-1 modulates human melanoma cell adhesion to laminin. Biochem. Biophys. Res. Commun. 209, 760 – 767.

30 van den Brule, F., Califice, S., Garnier, F., Fernandez, P. L., Berchuck, A. and Castronovo, V. (2003) Galectin-1 accumulation in the ovary carcinoma peritumoral stroma is induced by ovary carcinoma cells and affects both cancer cell proliferation and adhesion to laminin-1 and fibronectin. Lab. Invest. 83, 377 – 386.

31 Horiguchi, N., Arimoto, K.-i., Mizutani, A., Endo-Ichikawa, Y., Nakada, H. and Taketani, S. (2003) Galectin-1 induces cell adhesion to extracellular matrix and apoptosis of non-adherent human colon cancer Colo201 cells. J. Biochem. 134, 869 – 874.

32 Cooper, D. N. W., Massa, S. M. and Barondes, S. H (1991) Endogenous muscle lectin inhibits myoblasts adhesion to laminin. J. Cell Biol. 115, 1437 – 1448.

33 Gu, M., Wang, W., Song, W. K., Cooper, D. N. W. and Kaufman, S. J. (1994) Selective modulation of the interaction of α7β1 integrin with fibronectin and laminin by L-14 lectin during skeletal muscle differentiation. J. Cell Sci. 107, 175 – 181.

34 Rabinovich, G. A., Ariel, A., Hershkoviz, R., Hirabayashi, J., Kasai, K.-I. and Lider, O. (1999) Specific inhibition of T-cell adhesion to extracellular matrix and proinflammatory cytokine secretion by human recombinant galectin-1. Immunology 97, 100 – 106.

35 Moiseeva, E. P., Williams, B., Goodall, A. H. and Samani, N.J (2003) Galectin-1 interacts with β-1 subunit of integrin. Biochem. Biophys. Res. Commun. 310, 1010 – 1016.

36 Moiseeva, E. P., Spring, E. L., Baron, J. H. and de Bono, D. P. (1999) Galectin-1 modulates attachment, spreading and migration of cultured vascular smooth muscle cells via interactions with cellular receptors and components of extracellular matrix. J. Vasc. Res. 36, 47 – 58.

37 Martinez, V. G., Pellizzari, E. H., Díaz, E. S., Cigorraga, S. B., Lustig, L., Denduchis, B., Wolfenstein-Todel, C. and Iglesias, M. M. (2003) Galectin-1, a cell adhesion modulator, induces apoptosis of rat Leydig cells *in vitro*. Glycobiology 14, 127 – 137.

38 Andre, S., Kojima, S., Yamazaki, N., Fink, C., Kaltner, H., Kayser, K. and Gabius, H.-J. (1999) Galectins-1 and -3 and their ligands in tumor biology. Non-uniform properties in cell-surface presentation and modulation of adhesion to matrix glycoproteins for various tumor cell lines, in biodistribution of free and liposome-bound galectins and in their expression by breast and colorectal carcinoma with/without metastatic propensity. J. Cancer Res. Clin. Oncol. 125, 461 – 474.

39 Tinari, N., Kuwabara, I., Huflejt, M.E., Shen, P.F., Iacobelli, S.and Liu, F.-T. (2001) Glycoprotein 90 K/Mac-2BP interacts with galectin-1 and mediates galectin-1-induced cell aggregation. Int. J. Cancer 91, 167 – 172.

40 Rabinovich, G., Cumashi, A., Bianco, G. A., Ciavardelli, D., Iurisci, I., D'Egidio, M., Piccolo, M., Tinari, N., Nifantiev, N. and Iacobelli, S. (2006) Synthetic lactulose amines: novel class of anticancer agents that induce tumor cell apoptosis and inhibit galectin-mediated homotypic cell aggregation and endothelial cell morphogenesis. Glycobiology 16, 210 – 220.

41 Lotan, R., Belloni, P. N., Tressler, R. J., Lotan, D., Xu, X. C. and Nicolson, G. L. (1994) Expression of galectins on microvessel endothelial cells and their involvement in tumor cell adhesion. Glycoconj. J. 11, 462 – 468.

42 Glinsky, V. V., Huflejt, M. E., Glinsky, G. V., Deutscher, S. L. and Quinn, T. P. (2000) Effects of Thomsen-Friedenreich antigen-specific peptide P-30 on β-galactoside-mediated homotypic aggregation and adhesion to the endothelium of MDA-MB-435 human breast carcinoma cells. Cancer Res. 60, 2584 – 2588.

43 Baum, L. G., Pang, M., Perillo, N. L., Wu, T., Delegeane, A., Uittenbogaart, C. H., Fukuda, M. and Seilhamer, J. J. (1995) Human thymic epithelial cells express an endogenous lectin, galectin-1, which binds to core 2O-glycans on thymocytes and T lymphoblastoid cells. J. Exp. Med. 181, 877 – 887.

44 Friedl, P. (2004) Prespecification and plasticity: shifting mechanisms of cell migration. Curr. Opin. Cell Biol. 16, 14–23.

45 Camby, I., Belot, N., Lefranc, F., Sadeghi, N., de Launoit, Y., Kaltner, H., Musette, S., Darro, F., Danguy, A., Salmon, I. et al. (2002) Galectin-1 modulates human glioblastoma cell migration into the brain through modifications to the actin cytoskeleton and the levels of expression of small GTPases. J. Neuropathol. Exp. Neurol. 61, 585 – 597.

46 Camby, I., Le Mercier, M., Lefranc, F. and Kiss, R. (2006) Galectin-1: a small protein with major functions. Glycobiology 16, 137R-157R.

47 Camby, I., Decaestecker, C., Lefranc, F., Kaltner, H., Gabius, H.-J. and Kiss, R. (2005) Galectin-1 knocking down in human U87 glioblastoma cells alters their gene expression pattern. Biochem. Biophys. Res. Commun. 335, 27 – 35.

48 Maeda, N., Kawada, N., Seki, S., Arakawa, T., Ikeda, K., Iwao, H., Okuyama, H., Hirabayashi, J., Kasai, K.-i. and Yoshizato, K. (2003) Stimulation of proliferation of rat hepatic stellate cells by galectin-1 and galectin-3 through different intracellular signaling pathways. J. Biol. Chem. 278, 18938 – 18944.

49 Fukaya, K., Hasegawa, M., Mashitani, T., Kadoya, T., Horie, H., Hayashi, Y., Fujisawa, H., Tachibana, O., Kida, S. and Yamashita, J. (2003) Oxidized galectin-1 stimulates the migration of Schwann cells from proximal and distal stumps of transfected nerves and promotes axonal regeneration after peripheral nerve injury. J. Neuropathol. Exp. Neurol. 62, 162 – 172.

50 Alge, C. S., Priglinger, S. G., Kook, D., Schmid, H., Haritoglou, C., Welge-Lussen, U. and Kampik, A. (2006) Galectin-1 influences migration of retinal pigment epithelial cells. Invest. Ophthal. Vis. Sci. 47, 415 – 426.

51 Harvey, S., Zhang, Y., Landry, F., Miller, C. and Smith, J. W. (2001) Insights into a plasma membrane signature. Physiol. Genom. 5, 129 – 136.

52 Thijssen, V. L. J. L., Postel, R., Brandwijk, R. J. M. G. E., Dings, R. P. M., Nesmelova, Y., Satijn, S., Verhofstad, N., Nakabeppu, Y., Baum, L. G., Bakkers, J. et al. (2006) Galectin-1 is essential in tumor angiogenesis and is a target for antiangiogenesis therapy. Proc. Natl. Acad. Sci. 103, 15975 – 15980.

53 Clausse, N., van den Brule, F., Waltregny, D., Garnier, F. and Castronovo, V. (1999) Galectin-1 expression in prostate tumor-associated capillary endothelial cells is increased by prostate carcinoma cells and modulates heterotypic cell-cell adhesion. Angiogenesis 3, 317 – 325.

54 Fulcher, J. A., Hashimi, S. T., Levroney, E. L., Pang, M., Gurney, K. B., Baum, L. G. and Lee, B. (2006) Galectin-1-maturated human monocyte-derived dendritic cells have enhanced migration through extracellular matrix. J. Immunol. 177, 216 – 226.

55 Rabinovich, G. A., Sotomayor, C.E., Riera, C. M., Bianco, I. and Correa, S. G. (2000) Evidence of a role for galectin-1 in acute inflammation. Eur. J. Immunol. 30, 1331 – 1339.

56 La, M., Cao, T. V., Cerchiaro, G., Chilton, K., Hirabayashi, J., Kasai, K.-i., Oliani, S. M., Chernajovsky, Y. and Peretti, M. (2003) A novel biological activity for galectin-1. Inhibition of leukocyte-endothelial cell interactions in experimental inflammation. Am. J. Pathol. 163, 1505 – 1515.

57 Delbrouck, C., Doyen, I., Belot, N., Decaestecker, C., Ghanooni, R., de Lavareille, A., Kaltner, H., Choufani, G., Danguy, A., Vandenhoven, G. et al. (2002) Galectin-1 is overexpressed in nasal polyps under budesonide and inhibits eosinophil migration. Lab. Invest. 82, 147 – 158.

58 Hittelet, A., Legendre, H., Nagy, N., Bronckart, Y., Pector, J.C., Salmon, I., Yeaton, P., Gabius, H.-J., Kiss, R. and Camby, I. (2003) Upregulation of galectins-1 and -3 in human colon cancer and their role in regulating cell migration. Int. J. Cancer 103, 370 – 379.

59 Gil, C. D., Cooper, D., Rosignoli, G., Perretti, M. and Oliani, S. M. (2006) Inflammation-induced modulation of cellular galectin-1 and -3 expression in a model of rat peritonitis. Inflamm. Res. 55, 99 – 107.

60 He, J. and Baum, L. G. (2006) Endothelial cell expression of galectin-1 induced by prostate cancer cells inhibits T-cell transendothelial migration. Lab. Invest. 86, 578 – 590.

61 Gitt, M.A. and Barondes, S.H. (1991) Genomic sequence and organization of two members of a human lectin gene family. Biochemistry 30, 82 – 89.

62 Gitt, M. A., Massa, S. M., Leffler, H. and Barondes, S. H. (1992) Isolation and expression of a gene encoding L-14-II, a new human soluble lactose-binding lectin. J. Biol. Chem. 267, 10601 – 10606.

63 Saal, I., Nagy, N., Lensch, M., Lohr, M., Manning, J. C., Decaestecker, C., André, S., Kiss, R., Salmon, I. and Gabius, H.-J. (2005) Human gal-2: expression profiling by RT-PCR/ immunohistochemistry and its introduction as a histochemical tool for ligand localization. Histol. Histopathol. 20, 1191 – 1208.

64 Sturm, A., Lensch, M., André, S., Kaltner, H., Wiedenmann, B., Rosewicz, S., Dignass, A. U. and Gabius, H.-J. (2004) Human galectin-2: novel inducer of T cell apoptosis with distinct profile of caspase activation. J. Immunol. 173, 3825 – 3837.

65 Ozaki, K., Inoue, K., Sato, H., Iida, A., Ohnishi, Y., Sekine, A., Odashiro, K., Nobuyoshi, M., Hori, M. and Nakamura, Y. (2004) Functional variation in LSGAL2 confers risk of myocardial infarction and regulates lymphotoxin-alpha secretion *in vitro*. Nature 429, 72 – 75.

66 Kuwabara, Y. and Liu, F.-T. (1996) Galectin-3 promotes adhesion of human neutrophils to laminin. J. Immunol. 156, 3939 – 3944.

67 Hsu, D. K., Zuberi, R.I. and Liu, F.-T. (1992) Biochemical and biophysical characterization of human recombinant IgE-binding protein, an S-type animal lectin. J. Biol. Chem. 267, 14167 – 14174.

68 Massa, S. M., Cooper, D. N., Leffler, H. and Barondes, S. H. (1993) L-29, an endogenous lectin, binds to glycoconjugate ligands with positive cooperativity. Biochemistry 32, 260 – 267.

69 Warfield, P. R., Makker, P. N., Raz, A. and Ochieng, J. (1997) Adhesion of human breast carcinoma to extracellular matrix proteins is modulated by galectin-3. Invasion Metastasis 17, 101 – 112.

70 Ochieng, J., Warfield, P., Green-Jarvis, B. and Fentie, I. (1999) Galectin-3 regulates the adhesive interaction between breast carcinoma cells and elastin. J. Cell. Biochem. 75, 505 – 514.

71 Raz, A., Zhu, D., Hogan, V., Shan, N., Raz, T., Karkash, R., Pazerin, G. and Carmi, P.(1990) Evidence for the role of 34 kD galactoside-binding lectin in transformation and metastasis. Int. J. Cancer 46, 871 – 877.

72 Inufusa, H., Nakamura, M., Adachi, T., Aga, M., Kurimoto, M., Nakatani, Y., Wakano, T., Miyake, M., Okuno, K., Shiozaki, H. et al. (2001) Role of galectin-3 in adenocarcinoma liver metastasis. Int. J. Oncol. 19, 913 – 919.

73 Le Marer, N. and Hughes, R. C. (1996) Effects of the carbohydrate-binding protein galectin-3 on the invasiveness of human breast carcinoma cells. J. Cell. Physiol. 168, 51 – 58.

74 Ochieng, J., Gerold, M. and Raz, A. (1992) Dichotomy in the laminin binding properties of soluble and membrane bound human galactoside binding protein. Biochem. Biophys. Res. Commun. 186, 1674 – 1680.

75 Sato, S. and Hughes, R.C.(1992) Binding specificity of a baby hamster kidney lectin for H type I and II chains, polylactosamine glycans, and appropriately glycosylated forms of laminin and fibronectin. J. Biol. Chem. 267, 6983 – 6990.

76 Ochieng, J., Leite-Browning, M. L. and Warfield, P. (1998) Regulation of cellular adhesion to extracellular matrix proteins by galectin-3. Biochem. Biophys. Res. Commun. 246, 788 – 791.

77 Furtak, V., Hatcher, F. and Ochieng, J. (2001) Galectin-3 mediates the endocytosis of beta integrins by breast carcinoma cells. Biochem. Biophys. Res. Commun. 289, 845 – 850.

78 Inohara, H. and Raz, A. (1995) Functional evidence that cell surface galectin-3 mediates homotypic cell adhesion. Cancer Res. 55, 367 – 371.

79 Glinsky, V. V., Glinsky, G. V., Glinskii, O. V., Huxley, V. H., Turk, J. R., Mossine, V. V., Deutscher, S. L., Pienta, K. J. and Quinn, T. P. (2003) Intravascular metastatic cancer cell homotypic aggregation at the sites of primary attachment to the endothelium. Cancer Res. 63, 3805 – 3811.

80 Glinsky, V. V., Glinsky, G. V., Rittenhouse-Olson, K., Huflejt, M. E., Glinskii, O. V., Deutscher, S. L. and Quinn, T. P. (2001) The role of Thomsen-Friedenreich antigen in adhesion of human breast and prostate cancer cells to the endothelium. Cancer Res. 61, 4851 – 4857.

81 Grassadonia, A., Tinari, N., Iurisci, Y., Piccolo, E., Cumashi, A., Innominato, P., D'Egidio, M., Natori, C., Piantelli, M. and Iacobelli, S. (2004) 90 K (Mac-2 BP) and galectins in tumor progression and metastasis. Glycoconj. J. 19, 551 – 556.

82 Zou, J., Glinsky, V. V., Landon, L. A., Matthews, L. and Deutscher, S. L. (2005) Peptides speficic to the galectin-3 carbohydrate recognition domain inhibit metastasis-associated cancer cell adhesion. Carcinogenesis 26, 309 – 318.

83 Pienta, K. J., Naik, H., Akhtar, A., Yamazaki, K., Replogle, T. S., Lehr, J., Donat, T. L., Tait, L., Hogan, V. and Raz, A. (1995) Inhibition of spontaneous metastasis in rat prostate cancer model by oral administration of modified citrus pectin. J. Natl. Cancer Inst. 87, 348 – 353.

84 Nangia-Makker, P., Hogan, V., Honjo, Y., Baccarini, S., Tait, L., Bresalier, R. and Raz, A. (2002) Inhibition of human cancer cell growth and metastasis in nude mice by oral intake of modified citrus pectin. J. Natl. Cancer Inst. 94, 1854 – 1862.

85 Lehr, J. E and Pienta, K. J. (1998) Preferential adhesion of prostate cancer cells to a human bone marrow endothelial cell line. J. Natl. Cancer Inst. 90, 118 – 123.

86 Khaldoyanidi, S. K., Glinsky, V. V., Sikora, L., Glinskii, A. B., Mossine, V. V., Quinn, T. P., Glinsky, G. V. and Sriramarao, P. (2003) MDA-MB-435 human breast carcinoma cell homo- and heterotypic adhesion under flow conditions is mediated in part by Thomsen-Friedenreich antigen-galectin-3 interactions. J. Biol. Chem. 278, 4127 – 4134.

87 Krishnan, V., Bane, S. M., Kawle, P. D., Naresh, K. N. and Kalraiya, R. D.(2005) Altered melanoma cell surface glycosylation mediates organ specific adhesion and metastasis via lectin receptors on the lung vascular endothelium. Clin. Exp. Metastasis 22, 11 – 24.

88 Gil, C. D., La, M., Perretti, M. and Oliani, S. M. (2006) Interaction of human neutrophils with endothelial cells regulates the expression of endogenous proteins annexin 1, galectin-1 and galectin-3. Cell Biol. Intern. 30, 338 – 344.

89 Ochieng, J., Green, B., Evans, S., James, O. and Warfield, P. (1998) Modulation of the biologic functions of galectin-3 by matrix metalloproteinases. Biochim. Biophys. Acta 1379, 97 – 106.

90 Shekhar, M. P. V., Nangia-Makker, P., Tait, L., Miller, F. and Raz, A. (2004) Alterations in galectin-3 expression and distribution correlate with breast cancer progression. Functional analysis of galectin-3 in breast epithelial-endothelial interactions. Am. J. Pathol. 165, 1931 – 1941.

91 John, C. M., Leffler, H., Kahl-Knutsson, B., Svensson, I. and Jarvis, G. A. (2003) Truncated galectin-3 inhibits tumor growth and metastasis in orthopic nude mouse model of human breast cancer. Clin. Cancer Res. 9, 2374 – 2383.

92 Swarte, V. V., Mebius, R. E., Joziasse, D. H., Van den Eijnden, D. H. and Kraal, G. (1998) Lymphocyte triggering via L-selectin leads to enhanced galectin-3-mediated binding to dendritic cells. Eur. J. Immunol. 28, 2864 – 2871.

93 Sano, H. and Liu, F.-T. (2001) Galectins: another family of chemottractants? Mod. Asp. Immunobiol. 2, 4 – 6.

94 Sano, H., Hsu, D. K., Yu, L., Apgar, J. R., Kuwabara, I., Yamanaka, T., Hirashima, M. and Liu, F.-T. (2000) Human galectin-3 is a novel chemoattractant for monocytes and macrophages. J. Immunol. 165, 2156 – 2164.

95 Liu, F.-T. (2005) Regulatory roles of galectins in the immune response. Int. Arch. Allergy Immunol. 136, 385 – 400.

96 Camby, I., Belot, N., Rovire, S., Lefranc, F., Maurage, C. A., Lahm, H., Kaltner, H., Hadari, Y., Ruchoux, M. M., Brotchi, J. et al. (2001) Galectins are differentially expressed in supratentorial pilocytic astrocytomas, astrocytomas, anaplastic astrocytomas and glioblastomas, and significantly modulate tumor astrocyte migration. Brain Pathol. 11, 12 – 26.

97 O'Driscoll, L., Linehan, R., Liang, Y. H., Joyce, H., Oglesby, Y. and Clynes, M. (2002) Galectin-3 expression alters adhesion, motility and invasion in a lung cell line (DLKP), *in vitro*. Anticancer Res. 22, 3117 – 3126.

98 Fukushi, J., Makagiansar, Y. T. and Stallcup, W. B. (2004) NG2 proteoglycan promotes endothelial cell motility and angiogenesis via engagement of galectin-3 and $\alpha_3\beta_1$ integrin. Mol. Biol. Cell 15, 3580 – 3590.

99 Savino, W., Mendes-da-Cruz, D. A., Smaniotto, S., Silva-Monteiro, E. and Villa-Verde, D. M. S. (2004) Molecular mechanisms governing thymocyte migration: combined role of chemokines and extracellular matrix. J. Leukoc. Biol. 75, 951 – 961.

100 Dennis, J. W., Pawling, J., Cheung, P., Partridge, E. and Demetriou, M. (2002) UDP-N-acetylglucosaminyltransferase V (Mgat5) deficient mice. Biochim. Biophys. Acta 1573, 414 – 422.

101 Lagana, A., Goetz, J. G., Cheung, P., Raz, A., Dennis, J. W. and Nabi, I. R. (2006) Galectin binding to Mgat5-modified N-glycans regulates fibronectin matrix remodeling in tumor cells. Mol. Cell. Biol. 26, 3181 – 3193.

102 Maeda, N., Kawada, N., Seki, S., Arakawa, T., Ikeda, K., Iwao, H., Okuyama, H., Hirabayashi, J., Kasai, K.-i. and Yoshizato, K. (2003) Stimulation of proliferation of rat hepatic stellate cells by galectin-1 and galectin-3 through different intracellular signaling pathways. J. Biol. Chem. 278, 18938 – 18944.

103 Debray, C., Vereecken, P., Belot, N., Teillard, P., Brion, J. P., Pandolfo, M. and Pochet, R. (2004) Multifaceted role of galectin-3 on human glioblastoma cell motility. Biochem. Biophys. Res. Commun. 325, 1393 – 1398.

104 Oda, Y., Herrmann, J., Gitt, M. A., Turck, C. W., Burlingame, A. L., Barondes, S. H., and Leffler, H. (1993) Soluble lactose-binding lectin from rat intestine with two different carbohydrate-binding domains in the same peptide chain. J. Biol. Chem. 268, 5929 – 5939.

105 Chiu, M. L., Jones, J. C. R. and O'Keefe, E. J. (1992) Restricted tissue distribution of a 37-kDa possible adherens junction protein. J. Cell Biol. 119, 1689 – 1700.

106 Chiu, M. L., Parry, D. A. D., Feldman, S. R., Klapper, D. G. and O'Keefe, E. J. (1994) An adherens junction protein is a member of the family of lactose-binding lectins. J. Biol. Chem. 269, 31770 – 31776.

107 Danielsen, E. M. and Deurs, B. V. (1997) Galectin-4 and small intestinal brush border enzymes form clusters. Mol. Biol. Cell 8, 2241 – 2251.

108 Nio, J., Kon, Y. and Iwanaga, T. (2005) Differential cellular expression of galectin family mRNAs in the epithelial cells of the mouse digestive tract. J. Histochem. Cytochem. 53, 1323 – 1334.

109 Huflejt, M. E., Jordan, E. T., Gitt, M. A., Barondes, S. H. and Leffler, H. (1997) Striking different localization of galectin-3 and galectin-4 in human colon adenocarcinoma T84 cells. J. Biol. Chem. 272, 14294 – 14303.

110 Braccia, A., Villani, M., Immerdal, L., Niels-Christiansen, L.-L., Nystrom, B. T., Hansen, G. H. and Danielsen, E. M. (2003) Microvillar membrane microdomains exist at physiological temperature. Role of galectin-4 as lipid raft stabilizer revealed by superrafts. J. Biol. Chem. 278, 15679 – 15684.

111 Wasano, K. and Hirakawa, Y. (1995) Rat intestinal galactoside-binding lectin L-36 functions as a structural protein in the superficial squamous cells of the esophageal epithelium. Cell Tissue Res. 281, 77 – 83.

112 Madsen, P., Rasmussen, H. H., Flint, T., Gromov, P., Kruse, T. A., Honoré, B., Vorum, H. and Celis, J. E. (1995) Cloning, expression, and chromosome mapping of human galectin-7. J. Biol. Chem. 270, 5823 – 5829.

113 Magnaldo, T., Bernerd, F. and Darmon, M. (1995) Galectin-7, a human 14-kDa S-lectin, specifically expressed in keratinocytes and sensitive to retinoic acid. Dev. Biol. 168, 259 – 271.

114 Saussez, S. and Kiss, R. (2006) Galectin-7. Cell. Mol. Life Sci. 63, 686 – 697.

115 Polyak, K., Xia, Y., Zweier, J., Kinzler, K. and Vogelstein, B. (1997) A model for p-53-induced apoptosis. Nature 389, 300 – 305.

116 Kuwabara, I., Kuwabara, Y., Yang, R.-Y., Schuler, M., Green, D. R., Zuraw, B. L., Hsu, D. K. and Liu, F.-T. (2002) Galectin-7 (PIG1) exhibits pro-apoptotic function through JNK activation and mitochondrial cytochrome c release. J. Biol. Chem. 277, 3487 – 3497.

117 Demers, M., Magnaldo, T. and St-Pierre, Y. (2005) A novel function for galectin-7: promoting tumorigenesis by up-regulating MMP-9 gene expression. Cancer Res. 65, 5205 – 5210.

118 Cao, Z., Said, N., Amin, S., Wu, H. K., Bruce, A., Garate, M., Hsu, D.K., Kuwabara, I., Liu, F.-T. and Panjwani, N. (2002) Galectins-3 and -7, but not galectin-1, play a role in re-epithelialization of wounds. J. Biol. Chem. 277, 42299 – 42305.

119 Cao, Z., Said, N., Wu, H. K., Kuwabara, I., Liu, F.-T. and Panjwani, N. (2003) Galectin-7 as a potential mediator of corneal epithelial cell migration. Arch. Ophthalmol. 121, 82 – 86.

120 Hadari, Y. R., Paz, K., Dekel, R., Mestrovic, T., Accili, D. and Zick, Y. (1995) Galectin-8. A new rat galectin, related to gal-4. J. Biol. Chem. 270, 3447 – 3453.

121 Su, Z.-Z., Lin, J., Shen, R., Fisher, P. E., Goldstein, N. Y. and Fisher, P.B. (1996) Surface-epitope masking and expression cloning identifies the human prostate carcinoma tumor antigen PCTA-1 a member of the galectin gene family. Proc. Natl. Acad. Sci. 93, 7252 – 7257.

122 Bidon-Wagner, N. and Le Pennec, J.-P. (2004) Human galectin-8 isoforms and cancer. Glycoconj. J. 19, 557 – 563.

123 Nishi, N., Itoh, A., Fujiyama, A., Yoshida, N., Araya, S., Hirashima, M., Shoji, H. and Nakamura, T. (2005) Development of highly stable galectins: truncation of the linker peptide confers protease-resistance on tandem-repeat type galectins. FEBS Lett. 579, 2058 – 2064.

124 Nishi, N., Itoh, A., Shoji, H., Miyanaka, H. and Nakamura, T. (2006) Galectin-8 and galectin-9 are novel substrates for thrombin. Glycobiology 16, 15C-20C.

125 Levy, Y., Arbel-Goren, R., Hadari, Y. R., Eshhar, S., Ronen, D., Elhanany, E., Geiger, B. and Zick, Y. (2001) Galectin-8 functions as a matricellular modulator of cell adhesion. J. Biol. Chem. 276, 31285 – 31295.

126 Zick, Y., Eisenstein, M., Goren, R. A., Hadari, Y. R., Levy, Y. and Ronen, D. (2004) Role of galectin-8 as a modulator of cell adhesion and cell growth. Glycoconj. J. 19, 517 – 526.

127 Calof, A. L., Campanero, M. R., O'Rear, J. J., Yurchenco, P. D. and Lander, A. D. (1994) Domain-specific activation of neuronal migration and neurite outgrowth-promoting activities of laminin. Neuron 13, 117 – 130.

128 Yamada, K. M. and Kennedy, D. W. (1984) Dualistic nature of adhesive protein function: fibronectin and its biologically active peptide fragments can autoinhibit fibronectin function. J. Cell Biol. 99, 29 – 36.

129 Hadari, Y. R., Goren, R., Levy, Y., Amsterdam, A., Alon, R., Zakut, R. and Zick, Y. (2000) Galectin-8 binding to integrins inhibits cell adhesion and induces apoptosis. J. Cell Sci. 113, 2385 – 2397.

130 Levy, Y., Ronen, D., Bershadsky, A. D. and Zick, Y. (2003) Sustained induction of ERK, PKB and p70S6K regulates cell spreading and formation of F-actin microspikes upon ligation of integrins by galectin-8, a mammalian lectin. J. Biol. Chem. 278, 14533 – 14542.

131 Arbel-Goren, R., Levy, Y., Ronen, D. and Zick, Y. (2005) Cyclin-dependent kinase inhibitors and JNK act as molecular switches, regulating the choice between growth arrest and apoptosis induced by galectin-8. J. Biol. Chem. 280, 19105 – 19114.

132 Levy, Y., Auslender, S., Eisenstein, M., Vidavski, R. R., Ronen, D., Bershadsky, A. D. and Zick, Y. (2006) It depends on the hinge: a structure-functional analysis of galectin-8, a tandem-repeat type lectin. Glycobiology 16, 463 – 476.

133 Nishi, N., Shoji, H., Seki, M., Itoh, A., Miyanaka, H., Yuube, K., Hirashima, M. and Nakamura, T. (2003) Galectin-8 modulates neutrophil function via interaction with integrin αM. Glycobiology 13, 755 – 763.

134 Cárcamo, C., Pardo, E., Oyanadel, C., Bravo-Zehnder, M., Bull, P., Cáceres, M., Martínez, J., Massardo, L., Jacobelli, S., González, A. et al. (2006) Galectin-8 binds specific β1 integrins and induces polarized spreading highlighted by asymmetric lamellipodia in Jurkat T cells. Exp. Cell Res. 312, 374 – 386.

135 Nagy, N., Bronckart, Y., Camby, I., Legendre, H., Lahm, H., Kaltner, H., Hadari, Y., Van Ham, P., Yeaton, P., Pector, J.-C. et al. (2002) Galectin-8 expression decreases in cancer compared with normal and dysplastic human colon tissue and acts significantly on human colon cancer cell migration as a suppressor. Gut 50, 392 – 401.

136 Wada, J. and Kanwar, Y. S. (1997) Identification and characterization of galectin-9, a novel β-galactoside-binding mammalian lectin. J. Biol. Chem. 272, 6078 – 6086.

137 Asakura, H., Kashio, Y., Nakamura, K., Seki, M., Dai, S., Shirato, Y., Abedin, M. J., Yoshida, N. , Nishi, N., Imaizumi, T. et al. (2002) Selective eosinophil adhesion to fibroblast via IFN-γ-induced galectin-9. J. Immunol. 169, 5912 – 5918.

138 Irie, A., Yamauchi, A., Kontani, K., Kihara, M., Liu, D., Shirato, Y., Seki, M., Nishi, N., Nakamura, T., Yokomise, H. et al. (2005) Galectin-9 as a prognostic factor with antimetastatic potential in breast cancer. Clin. Cancer Res. 11, 2962 – 2968.

139 Kasamatsu, A., Uzawa, K., Nakashima, D., Koike, H., Shiiba, M., Bukawa, H., Yokoe, H. and Tanzawa, H. (2005) Galectin-9 as a regulator of cellular adhesion in human oral squamous cell carcinoma cell lines. Int. J. Mol. Med. 16, 269 – 273.

140 Imaizumi, T., Kumagai, M., Nishi, N., Hirashima, M., Hatakeyama, M., Tamo, W., Yoshida, H., Nakamura, T., Okumura, K. and Satoh, K. (2003) 15-Deoxy-delta(12,14)-prostaglandin J2 inhibits IFN-gamma-induced galectin-9 expression in cultured human umbilical vein endothelial cells. Int. Arch. Allergy Immunol. 131, 57 – 61.

141 Ishikawa, A., Imaizumi, T., Yoshida, H., Nishi, N., Nakamura, T., Hirashima, M. and Satoh, K. (2004) Double-stranded RNA enhances the expression of galectin-9 in vascular endothelial cells. Immunol. Cell Biol. 82, 410 – 414.

142 Matsumoto, R., Hirashima, M., Kita, H. and Gleich, G. J. (2002) Biological activities of ecalectin: a novel eosinophil-activating factor. J. Immunol. 168, 1961 – 1967.

143 Kageshita, T., Kashio, Y., Yamauchi, A., Seki, M., Abedin, M. J., Nishi, N., Shoji, H., Nakamura, T., Ono, T. and Hirashima, M. (2002) Possible role of galectin-9 in cell aggregation and apoptosis of human melanoma cell lines and its clinical significance. Int. J. Cancer 99, 809 – 816.

144 Zhu, C., Anderson, A. C., Schubart, A., Xiong, H., Imitola, J., Khoury, S. J., Zheng, X. X., Strom, T. B. and Kuchroo, V. K. (2005) The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity. Nat. Immunol. 6, 1245 – 1252.

145 Matsumoto, R., Matsumoto, H., Seki, M., Hata, M., Asano, Y., Kanegasaki, S., Stevens, R. L. and Hirashima, M. (1998) Human ecalectin, a variant of human galectin-9, is a novel eosinophil chemoattractant produced by T lymphocytes. J. Biol. Chem. 273, 16976 – 16984.

146 Matsushita, N., Nishi, N., Seki, M., Matsumoto, R., Kuwabara, Y., Liu, F.-T., Hata, Y., Nakamura, T. and Hirashima, M. (2000) Requirement of divalent galactoside-binding activity of ecalectin/galectin-9 for eosinophil chemoattraction. J. Biol. Chem. 275, 8355 – 8360.

147 Hirashima, M. (2000) Ecalectin/galectin-9, a novel eosinophil chemoattractant: its function and production. Int. Arch. Allergy Immunol. 122, 6 – 9.

148 Sato, M., Nishi, N., Shoji, H., Seki, M., Hashidate, T., Hirabayashi, J., Kasai, K.-i., Hata, Y., Suzuki, S., Hirashima, M. et al. (2002) Functional analysis of the carbohydrate recognition domains and a linker peptide of galectin-9 as to eosinophil chemoattractant activity. Glycobiology 12, 191 – 197.

149 Chabot, S., Kashio, Y., Seki, M., Shirato, Y., Nakamura K, Nishi, N., Nakamura, T., Matsumoto, R. and Hirashima, M. (2002) Regulation of galectin-9 expression and release in Jurkat T cell line cells. Glycobiology 12, 111 – 118.

150 Gitt, M. A., Colnot, C., Poirier, F., Nani, K. J., Barondes, S. H. and Leffler, H. (1998) Galectin-4 and galectin-6 are two closely related lectins expressed in mouse gastrointestinal tract. J. Biol. Chem. 273, 2954 – 2960.

151 Gray, C. A., Dunlap, K. A., Burghardt, R. C. and Spencer, T. E. (2005) Galectin-15 in ovine uteroplacental tissues. Reproduction 130, 231 – 240.

152 Spencer, T. E., Johnson, G. A., Bazer, F. W. and Burghardt, R. C. (2004) Implantation mechanisms: insights from the sheep. Reproduction 128, 657 – 668.

153 Yu, L.G., Andrews, N., Zhao, Q., McKean, D., Williams, J. F., Connor, L. J., Gerasimenko, O. V., Hilkens, J., Hirabayashi, J., Kasai, K. et al. (2007) Galectin-3 interaction with Thomsen-Friedenreich disaccharide on cancer-associated MUC1 causes increased cancer cell endothelial adhesion. J. Biol. Chem. 282, 773 – 781.

154 Lahm, H., André, S., Hoeflich, A., Fisher, J. R., Sordat, B., Kaltner, H., Wolf, E. and Gabius, H.-J. (2001) Comprehensive galectin fingerprinting in a panel of 61 human tumor cell lines by RT-PCR and its implications for diagnostic and therapeutic procedures. J. Cancer Res. Clin. Oncol. 127, 375 – 386.

155 Talts, J. F., Andac, Z., Gohring, W., Brancaccio, A. and Timpl, R. (1999) Binding of the G domains of laminin α1 and α2 chains and perlecan to heparin, sulfatides, α-dystroglycan and several extracellular matrix proteins. EMBO J. 18, 863 – 870.

156 Sánchez-Aparicio, P., Domínguez-Jimenez, C. and Garcia-Pardo, A. (1994) Activation of the $\alpha_4\beta_1$ integrin through the β_1 subunit induces recognition of the RGDS sequence in fibronectin. J. Cell Biol. 126, 271 – 279.

157 Glinsky, G. V. (1998) Anti-adhesion cancer therapy. Cancer Metastasis Rev. 17, 177 – 185.

158 Meromsky, L., Lotan, R. and Raz, A. (1986) Implications of endogenous tumor cell surface lectins as mediators of cellular interactions and lung colonization. Cancer Res. 46, 5270 – 5275.

159 Inohara, H. and Raz, A. (1994) Effects of natural complex carbohydrate (citrus pectin) on murine melanoma cell properties related to galectin-3 functions. Glycoconj. J. 11, 527 – 532.

160 Glinsky, G. V., Price, J. E., Glinsky, V. V., Mossine, V. V., Kiriakova, G. and Metcalf, J. B. (1996) Inhibition of human breast cancer metastasis in nude mice by synthetic glyco-amines. Cancer Res. 56, 5319 – 5324.

161 Rubinstein, N., Alvarez, M., Zwirner, N. W., Toscano, M. A., Ilarregui, J. M., Bravo, A., Mordoh, J., Fainboim, L., Podhajcer, O. L. and Rabinovich, G. A. (2004) Targeted inhibition of galectin-1 gene expression results in heightened T cell-mediated rejection: a potential mechanism of tumor-immune privilege. Cancer Cell 5, 241 – 251.

162 Lefranc, F., Brotchi, J. and Kiss, R. (2005) Possible future issues in the treatment of glioblastomas: special emphasis on cell migration and the resistance of migrating glioblastoma cells to apoptosis. J. Clin. Oncol. 23, 2411 – 2422.

163 Ingrassia, L., Nshimyumukiza, P., Dewelle, J., Lefranc, F., Wlodarczak, L., Thomas, S., Dielie, G., Chiron, C., Zedde, C., Tisnes, P., van Soest, R., Braekman, J.-C., Darro, F. and Kiss, R. (2006) A lactosylated steroid contributes in vivo therapeutic benefits in experimental models of lymphoma and human glioblastoma. J. Med. Chem. 49, 1800 – 1807.