Biochimica et Biophysica Acta 1836 (2013) 177–185

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbacan

Review

Galectin-9 in tumor biology: A jack of multiple trades ☆

Roy Heusschen [1], Arjan W. Griffioen, Victor L. Thijssen *

Department of Medical Oncology, VU University Medical Center, Amsterdam, The Netherlands

ARTICLE INFO

Article history:
Received 5 March 2013
Received in revised form 25 April 2013
Accepted 29 April 2013
Available online 4 May 2013

Keywords:
Galectin
Metastasis
Immune escape
Angiogenesis
Cancer therapy

ABSTRACT

Galectin family members have been shown to exert multiple roles in the context of tumor biology. Several recent findings support a similar multi-faceted role for galectin-9. Galectin-9 expression is frequently altered in cancer as compared to normal tissues. In addition, an increasing amount of evidence suggests that galectin-9 is involved in several aspects of tumor progression, including tumor cell adhesion and survival, immune escape and angiogenesis. Also, galectin-9 shows potential as a prognostic marker and a therapeutic target for several malignancies. In this review we summarize both the established and the emerging roles of galectin-9 in tumor biology and discuss the potential application of galectin-9 in anti-cancer therapy.

Contents

1. Introduction

Galectins are characterized by β-galactoside binding affinity and the presence of an evolutionary conserved sequence: the carbohydrate recognition domain [1,2] (CRD). To date, 15 mammalian galectins have been identified which can be subdivided into 3 groups. Galectin-3, which contains a proline–glycine rich N-terminal tail fused to a CRD, is classified as a chimeric galectin. All other galectin family members either contain one CRD, i.e. prototype galectins, or 2 CRDs which are linked by a linker domain, i.e. tandem-repeat galectins (Fig. 1). Galectins decode and interpret the glycome code by binding to specific carbohydrate moieties on glycoconjugates. Since galectins can di- or multimerize, their interaction with carbohydrates enables them to cluster and cross-link multivalent glycoconjugates, resulting in a large degree of diversity in terms of specificity, affinity and valency for carbohydrates within the galectin family [3–5]. This allows galectins that are present in the extracellular environment to influence cell–cell and cell–matrix interactions and to modulate signaling pathways by regulating receptor lattice formation on the cell membrane. The carbohydrate binding activity is also required for secretion of galectins since they lack a classical secretion signal [5,6].

Next to their extracellular localization and function, galectins are also found intracellularly, both in the cytoplasm and the nucleus. There, they have been implicated in processes like signal transduction

☆ This work was supported by grants from the Dutch Cancer Society (UM2008-4101; VU2009-4358) to AWG and VLT.
* Corresponding author at: Angiogenesis Laboratory Amsterdam, Department of Medical Oncology, VU University Medical Center, De Boelelaan 1118, 1081 HV Amsterdam, The Netherlands. Tel.: +31 20 4442406; fax: +31 20 4443844.
E-mail address: v.thijssen@vumc.nl (V.L. Thijssen).
[1] Current affiliation: Laboratory of Hematology, GIGA-Research, University of Liège, Liège, Belgium.

0304-419X/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbcan.2013.04.006

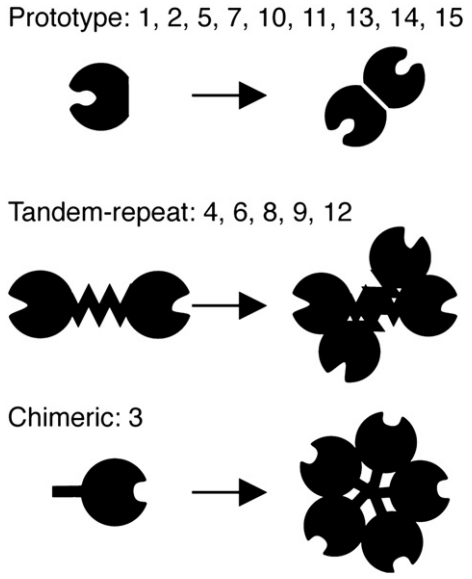

Fig. 1. The galectin protein family. Schematic representation of the different subgroups within the galectin protein family. Prototype galectins consist of a single CRD, tandem repeat galectins have two CRDs joined by a linker peptide and chimeric galectins have tail of short amino acid repeats at the N-terminal side of the CRD. Galectin dimerization and oligomerization increases binding valency which is important for galectin function.

and mRNA splicing which are mainly carbohydrate independent [2,7,8]. The widespread localization of galectins implicates that they exert a large variety of functional roles. Indeed, galectins have been shown to participate in physiological processes including brain development [9,10], angiogenesis [11–13], T cell homeostasis [4,5,14] and feto-maternal tolerance [15,16]. In addition, galectins have been associated with different pathologies, most notably with cancer [7]. In fact, galectins contribute to different key events during tumorigenesis. For example, galectins can mediate tumor cell transformation by interacting with oncogenic Ras [7,17,18] and affect tumor cell survival [19,20]. Galectins have also been described to modulate homo- and heterotypic adhesion of tumor cells thereby mediating tumor metastasis [7,21,22]. Finally, different galectin family members have been implicated in tumor immune escape [23,24] and tumor angiogenesis [11,13,19]. In recent years, an increasing amount of data suggests a similar multi-faceted role for galectin-9 in tumor biology. Here, we summarize the established and hypothesized roles of galectin-9 in tumor biology, and discuss its potential as a therapeutic target for cancer treatment.

2. Galectin-9: Gene, protein, and binding partners

The cDNA of galectin-9, a tandem-repeat galectin, was cloned in 1997 by 3 independent groups working in different research areas [25–27]. The galectin-9 gene, i.e. *LGALS9* (HGNC:6570), is located on chromosome 17q11.2 and consists of 11 exons encoding a 355 amino acid long protein of approximately 39.5 kD. In humans, two additional *LGALS9*-like genes have been described, *LGALS9B* (HGNC:24842) and *LGALS9C* (HGNC:33874) [2], which are both located on chromosome 17p11.2 close to LGALS9 which is indicative of gene duplication (Fig. 2A). Both have a good exon–intron structure but whether these genes are transcriptionally active is still under debate. Matsushita et al. claim, based on in silico comparison of cloned galectin-9 sequences, that all transcripts found are derived from a single gene, i.e. *LGALS9* [28]. On the other hand, other groups reported that *LGALS9B* and *LGALS9C* are transcriptionally active and protein-coding [29–31]. Given the high percentage of similarity between the galectin-9 derived proteins (Fig. 2B) it remains elusive whether they can exert different cellular functions.

A feature that has been shown to affect galectin-9 protein function is posttranscriptional splicing, a phenomenon which has also been described for galectin-8, another tandem-repeat galectin [32–34]. In the case of galectin-9, splice variants vary in the exclusion of exons 5, 6 and 10 from the full length (FL) mRNA transcript. Three splice variants are frequently described in the literature, i.e. gal-9FL (or gal-9L), gal-9Δ5 (or gal-9M, ecalectin) and gal-9Δ5/6 (or gal-9S) [35]. In addition, several groups now reported splice variants lacking exon 10, i.e. gal-9Δ10, gal-9Δ5/10 and gal-9Δ5/6/10 [16,36]. Exclusion of exon 10 from the mature mRNA transcript generates a frameshift and premature stop codon within exon 11, resulting in a truncation of the C-terminal CRD and a prototype-like galectin (Fig. 2C). The function of such a truncated galectin-9 is still unresolved. Exclusion of exon 5, alone or in combination with exon 6, results in length variations of the linker domain between the two CRDs. This linker domain influences the rotational freedom of both CRDs and mediates higher-order multimer formation thereby increasing gal-9 valency [37,38]. As a result, splice variants varying in linker length likely have different specificities and/or affinities for glycoconjugates. Splicing also appears to affect the cellular localization of galectin-9. The protein has been found extracellularly as well as intracellularly, both in the nucleus and in the cytoplasm [39,40] (Fig. 2D). Interestingly, splice variants lacking exon 10 do not seem to be secreted [41], suggesting an intracellular role for these proteins. While this function has not been identified yet, most intracellular galectins act through direct protein–protein interactions. It is thus likely that this is also the case for the intracellular galectin-9 isoforms. Different from the Δ10 isoform, secretion of other galectin-9 isoforms has been confirmed for various cell types, including T lymphocytes [35] and fibroblasts [42]. Although the precise mechanism by which galectin-9 is secreted is still not known, matrix-metalloproteinases (MMPs) and protein kinase C (PKC) appear to be involved [35]. Extracellularly, galectin-9 can regulate cell adhesion and lattice formation of glycoconjugates via its CRDs (Fig. 2D). The ligand binding is well regulated and dynamic. As already mentioned, variations in the linker length can affect valency. Furthermore, the specificity and affinity of the N- and C-terminal CRDs for specific glycoconjugates vary [43,44]. This readily affects the affinity for different binding partners. So far, a limited number of galectin-9 binding partners have been identified and verified (Table 1). For example, Tim-3 and CD44 have been identified as extracellular receptors for galectin-9 [45,46] and NF-IL6, a transcription factor, has been implicated as an intracellular binding partner [47]. Up to now, little is known regarding the binding of specific ligands to the different galectin-9 isoforms. In addition, the knowledge about the functional consequence of galectin-9 binding to binding partners is limited and a genome-wide analysis to identify galectin-9 interacting proteins would enhance our understanding of the role and function of galectin-9.

3. Galectin-9 expression in cancer

Galectin-9 expression is widely distributed in tissues involved in the immune system, i.e. spleen, thymus and peripheral blood lymphocytes, and in tissues of endodermal origin, i.e. liver, intestine, stomach and lung. Conflicting data were reported for skeletal and cardiac muscles, brain and kidney [25–27] but this most likely reflects differences in the experimental setup and sensitivity of the assays used to detect galectin-9 expression. This is exemplified by the observation that gal-9FL – long thought to be an intestine specific isoform of galectin-9 – has now also been shown to be expressed in several other cell types, like immune cells and endothelial cells [35,40].

Regarding the expression in cancer, one of the groups that first cloned galectin-9 identified it as a tumor antigen in Hodgkin's lymphoma, implicating a role in tumor biology [25]. Indeed, several groups have now shown that galectin-9 levels vary when comparing tumor tissue to normal tissue (Table 2). Thus far, most of the studies

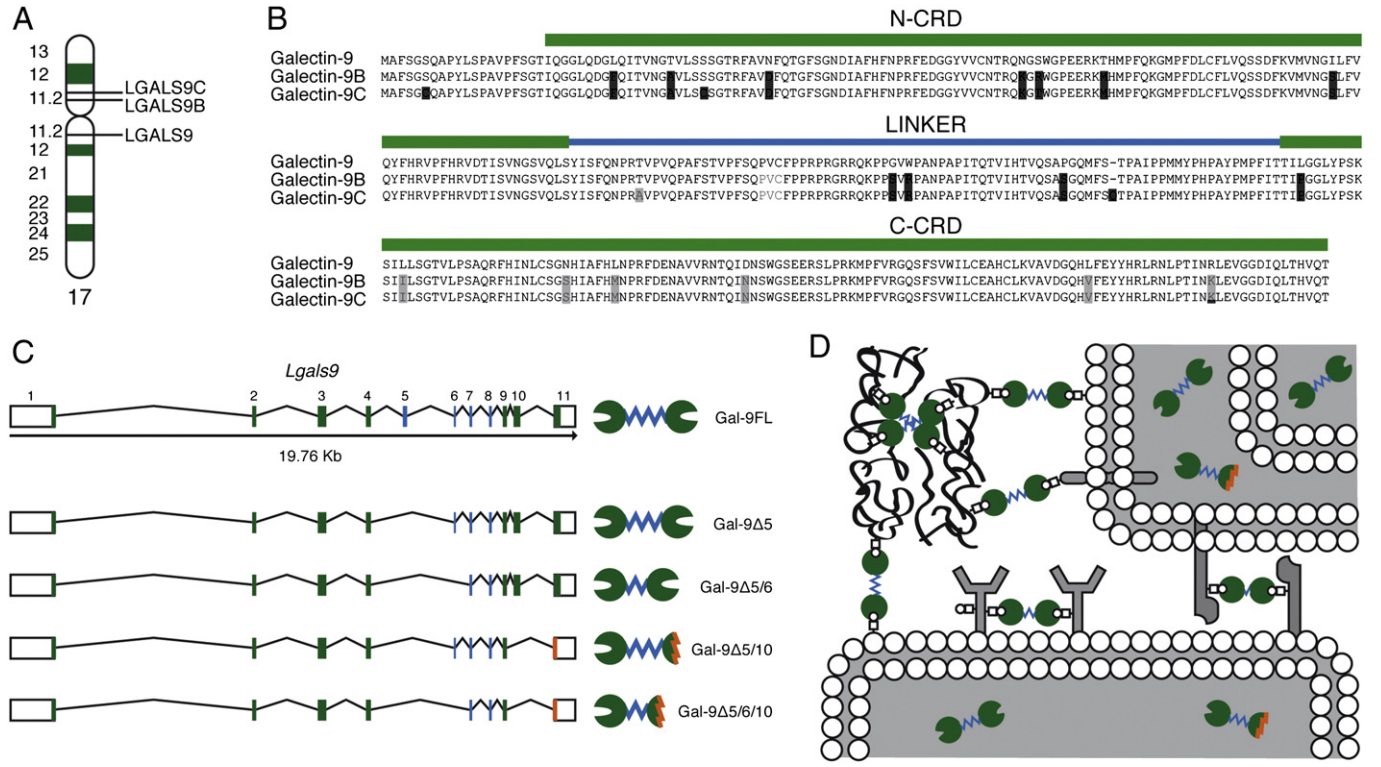

Fig. 2. Galectin-9 splice variants and protein localization. (A) Location of galectin-9 (*LGALS9*) and galectin-9-like (*LGALS9B* and *LGALS9C*) genes on chromosome 17. (B) Homology of LGALS9, LGALS9B and LGALS9C proteins. Gray boxes depict differences with LGALS9. Sequences encoding the CRDs and linker region have green and blue bars above them respectively. (C) Galectin-9 (*LGALS9*) gene structure and confirmed splice variants. Boxes represent exons (white: untranslated region; green: CRD coding region; blue: linker coding region; and orange: frameshift in the coding region). So far, 6 splice variants have been identified that vary in the exclusion of exons 5, 6 and 10 [16,36]. As a result, linker length and the C-terminal CRD varies between splice variants. Gal-9FL: galectin-9 full length. (D) Protein localization of galectin-9. Galectin-9 can be found both intra- and extracellularly. Extracellularly galectin-9 can multimerize and is involved in adhesion of cells to the extracellular matrix and hetero- and homotypic cell adhesion via binding to varying carbohydrate epitopes (colored red, yellow and clear blue). In addition, galectin-9 can likely regulate receptor lattice formation and domain organization on the cell membrane. Galectin-9 is also found intracellularly, although its mode of action there is not clear yet.

show a low or decreased expression of galectin-9 in tumor cells as compared to their normal counterparts. For example, Lahm et al. performed an mRNA profiling study in an extensive panel of human tumor cell lines [48]. Breast, lung, melanoma, renal, adrenal and prostate cancer cell lines all showed low or absent galectin-9 expression. This is in line with other studies in which the expression, on both the mRNA and protein levels, appears to be lost during the transformation to a malignant phenotype [49–51]. A high or increased galectin-9 expression has only been reported in leukemia and colon cancer cell lines [48]. For cell lines derived from tumors of the nervous system and the ovaries, the expression of galectin-9 varied depending on the particular subtype of the tumor cell line investigated [48].

Interestingly, galectins are increasingly recognized as prognostic markers for malignancies [52,53] and galectin-9 is no exception to this (Table 2). When screening a panel of 84 breast tumors, Irie et al. found galectin-9 to be expressed in half of these tumors. Moreover, galectin-9 expression predicted distant metastasis better than lymph node status: 19 out of 21 metastasis-positive cases were galectin-9 negative while high galectin-9 expression resulted in an increased cumulative disease free survival of these patients [50]. Similar results were obtained in melanoma, cervical squamous cell carcinoma and hepatocellular carcinoma [51,54,55]. Altogether, most current data suggest an inverse relation between galectin-9 expression and cancer progression for the majority of solid tumors. However, it is important to note that most studies did not take into account the extensive galectin-9 splicing. Only Lahm et al. made a distinction between gal-9FL and gal-9Δ5, indicating that for a minority of cases these splice variants are differentially expressed in tumor cell lines [48,56]. It remains to be determined whether the expression of specific splice variants is also different in human tumor tissues. Furthermore, it is still largely unknown how the expression of galectin-9 is regulated. So far, interferon-gamma (IFNg) has been shown to induce galectin-9 expression in fibroblasts [42] and endothelial cells [57]. In the latter cells, IFNg-dependent expression and membrane translocation were under control of HDAC3 [58]. Additional modulators of galectin-9 include interleukin-1β (IL-1β) and interleukin-5 (IL-5) in respective astrocytes [59] and eosinophils [60]. Again, it remains to be studied whether and how these compounds affect galectin-9 isoform expression.

4. Functions of galectin-9 in cancer progression

It is now well established that individual galectins can modulate several processes during tumorigenesis simultaneously [7]. For example, galectin-1 mediates tumor cell transformation by anchoring oncogenic H-Ras to the membrane, resulting in excessive signaling [18]. In addition, galectin-1 modulates tumor cell adhesion and metastasis [61–63], tumor angiogenesis [13,64] and tumor immune escape [24]. Similarly, galectin-9 has now also been implicated in several aspects of cancer progression.

4.1. Apoptosis and cell cycle control

Galectins have classically been associated with cell-cycle control and apoptosis [20,65]. In tumor biology this is the most extensively studied property of galectins [7]. Aside from galectin-1 and galectin-3, galectin-9 also modulates cell survival. Galectin-9 induces apoptosis when added to various cell types, e.g. human melanoma cell lines, T cell lines and different types of leukemia cell lines [51,60,66–68]. This pro-apoptotic effect of galectin-9 is dependent

Table 1
Galectin-9 binding partners.

Binding partner	Carbohydrate dependent	Functional consequence	Reference
Verified			
Branched N-glycan-type oligosaccharides	Yes	ND	[43]
Linear b1-3-linked poly-N-acetyllactosamines	Yes	ND	[43]
Branched N-glycans without a2,3-sialylation	Yes	N-terminal CRD specific	[107]
A2,3-sialylated oligosaccharides	Yes	C-terminal CRD specific	[43]
Lipid rafts	ND	Induction osteoblast differentiation	[108]
Latent membrane protein 1	ND	ND	[109]
(Galb1-3)n epitopes	Yes	*Leishmania major* and host interaction	[110]
T cell immunoglobulin mucin-3 (Tim-3)	Yes	Th1 cell apoptosis modulation of numerous immune cell types	[45,99]
Thrombin	ND	Cleavage of gal-9 and decrease of eosinophil attraction	[111]
Galectin-9	Yes	Homo-multimerization and increased valency	[112]
Galectin-8	Yes	Hetero-multimerization and increased valency	[112]
Galectin-3	Yes	Hetero-multimerization and increased valency	[112]
CD44	Yes	Suppression of CD44-hyalunoric acid binding CD44/BMP receptor complex formation	[46] [108]
IgE	Yes	Prevention of mast cell degranulation	[113]
NF-IL6 (C/EBPb)	ND	Inflammatory cytokine production	[47]
Protein disulfide isomerases	Yes	Regulation of T-cell migration	[88]
Implicated			
Integrin-a4/b1 (ITGa4ba)	ND	Suppression of ITGa4b1/VCAM-1 binding	[74]
Vascular cell adhesion molecule-1 (VCAM-1)	ND	Suppression of ITGa4b1/VCAM-1 binding	[74]

on β-galactoside binding activity and signaling seems to occur through NF-κB and the calcium–calpain–caspase-1 pathway in T cells [67]. The receptor for galectin-9-mediated apoptosis on T-cells appears to be TIM-3 [45] although there are reports indicating that other receptors might be involved [69]. The observation that galectin-9 induces apoptosis of many blood cancer cells appears to contrast with the reports that these cells have an increased galectin-9 expression. However, for other galectins differential effects on cellular processes like proliferation and apoptosis were found to depend on the concentration and localization of the galectin [64,70,71]. It is likely that these context-dependent effects also apply to galectin-9. For example, in endothelial cells IFNg induces both a cell cycle arrest [72] and increased expression of galectin-9 [57]. This might indicate an inverse relation between galectin-9 and cell proliferation. Consequently, loss of galectin-9, as observed in many malignant cells, could simultaneously confer resistance to apoptosis and increase the proliferative potential. However, the supporting data are largely indirect and further studies are required to establish whether such a link exists.

4.2. Adhesion, migration and metastasis

Initial observations in tumor cell lines pointed towards the involvement of galectin-9 in tumor cell adhesion. For example, galectin-9 expressing melanoma cells form colonies while cells that lack galectin-9 expression do not [51]. Similarly, MCF7 breast cancer cells with high galectin-9 expression form clusters which is not observed for low galectin-9 expressing cells [50]. In the latter study it was shown that restoring galectin-9 expression also restored cell cluster formation. This was seen for gal-9FL, gal-9Δ5 and gal-9Δ5/6. Interestingly, increased expression of only gal-9FL and gal-9Δ5/6 inhibited the adhesion of MCF7 cells to different extracellular matrix components, including collagen, fibronectin, vitronectin and laminin [50]. On the other hand, low

Table 2
Galectin-9 expression and prognostic value in cancer.

Cancer type	Origin (a)	Protein/mRNA	Prognosis (b)	Localization (c)	Reference
Low or decreased galectin-9 expression					
Breast	Patient tissue	Protein	↑	C	[50]
	MCF7	protein	(↑)	C	[50]
	cell lines (9)	mRNA	ND	NS	[48]
Lung	Cell lines (10)	mRNA	ND	NS	[48]
Renal	ACHN	mRNA	ND	NS	[48]
Adrenal	SW13	mRNA	ND	NS	[48]
Prostate	Cell lines (3)	mRNA	ND	NS	[48]
Skin	Patient tissue	Protein	↑	C, N	[51]
	cell lines (3)	protein/mRNA	(↑)	C, M	[51]
	HS294T	mRNA	ND	NS	[48]
Cervical	Patient tissue	Protein	(↑)	C	[54]
Oral	Cell lines (3)	Protein/mRNA	(↑)	C	[49]
Brain	Cell lines (8)	mRNA	ND	NS	[48]
Ovarian	Cell lines (2)	mRNA	ND	NS	[48]
Liver	Patients	Protein	↑	C	[55]
High or increased galectin-9 expression					
Lymphoma	Patient tissue	Protein/mRNA	ND	NS	[25]
Leukemia	Cell lines (3)	mRNA	ND	NS	[48]
Colon	Cell lines (23)	mRNA	ND	NS	[48]

(a) Number of different cell lines tested is indicated between brackets, for additional information see the corresponding references. For cell lines, the classification was based on the expression level reported in the majority of cell lines. (b) ↑ = high expression is correlated with a good prognosis. (↑) = high expression is correlated with decreased aggressiveness, i.e. metastasis. ND = not determined; (c) C = cytoplasm; N = nucleus; M = membrane; and NS = not specified.

galectin-9 expressing oral squamous carcinoma cells (OSCCs) showed increased adhesion to collagen and fibronectin following overexpression of galectin-9 [49]. Unfortunately, the latter study did not state which galectin-9 variant was overexpressed. Possibly, the adhesion depends on the relative abundance of individual galectin-9 splice variants. Thus, while increased levels of gal-9FL and gal-9Δ5/6 seem to result in an inhibition of cell adhesion to extracellular matrix components, high abundance of gal-9Δ5 might promote it. Future studies could provide evidence whether or not such variant-dependent regulation of adhesion does occur. Some recent results provided insight that such a link might exist. Zhang et al. showed that increased expression of galectin-9 modulates E-selectin levels in LoVo colon carcinoma cells [73]. Interestingly, specific galectin-9 splice variants showed a diverging effect. Gal-9FL overexpression induces downregulation of E-selectin expression in these cells. Gal-9Δ5 and gal-9Δ5/6 overexpression results in the upregulation of E-selectin, with concomitant increased adhesion of these cells to endothelial cells. Whether this is a mechanism acting in vivo thereby facilitating colon cancer cells to metastasize remains to be determined. In addition, while gal-9FL overexpression did not result in an increased adhesion of LoVo cells to endothelial cells it did result in an increased adhesion of these cells to matrigel. This further illustrates that the effects of galectin-9 on adhesion depend on which ligands are available in a given setting. Since galectin-9 is secreted by various cell types [35,42], increased expression of this protein in cancer cell lines likely results in an increased secretion accounting for the observed effects. Indeed, extracellular galectin-9 has been implicated in the regulation of cell adhesion and metastasis. For example, exogenous galectin-9 induces aggregation and apoptosis of MM-RU cells [51]. In addition, intravenous administration of a stable recombinant galectin-9 lacking the linker region prevents lung metastasis of B16F10 and colon26 colon cancer cells [74]. Interestingly, both these cell lines highly express CD44 and integrin family members. Galectin-9 suppressed the binding of hyaluronic acid to CD44 in both cell lines and also suppressed the binding of vascular cell adhesion molecule-1 (VCAM-1) to integrin-α4β1 (ITGα4β1; very late antigen-4) in B16F10 cells. In this study, a stable form of recombinant human galectin-9 was used that lacks the linker domain. However, experiments performed with mouse gal-9Δ5 yielded similar results as those performed with the stable form of human galectin-9, suggesting the latter indeed reflects gal-9Δ5. However, further studies to confirm this are required. Of note, modulating cell adhesion by integrin binding and regulation is a recurrent theme in galectin biology, with different galectins regulating different integrins [75,76]. In fact, targeting of integrin/galectin axes shows promise as a diagnostic and therapeutic tool [77,78].

As already described, loss of galectin-9 expression is consistently correlated with increased tumor aggressiveness. In fact, in both melanoma and breast cancer patients, distant metastasis showed a lack of galectin-9 expression in the vast majority of cases [50,51] and galectin-9 expression in cervical squamous cell carcinoma and hepatocellular carcinoma is inversely correlated with malignant potential [54,55]. Altogether, current data suggest that the most dominant galectin-9 isoform, i.e. gal-9Δ5, might prevent metastasis by maintaining tissue integrity and hampering tumor cell migration and extravasation, while other galectin-9 isoforms facilitate metastasis. As a result, loss of gal-9Δ5 during tumor progression would result in increased aggressiveness. This was recently supported by findings of Zhang et al. who showed that loss of galectin-9 in hepatocellular carcinoma cells in vitro increased the adhesive and invasive activity of these cells [55].

4.3. Immune escape

Ever since its initial cloning, galectin-9 has been extensively studied in the context of immunity and inflammation [4,39]. Galectin-9 acts as a cytokine and modulates the activity and function of numerous types of immune cells. Surprisingly, a role for galectin-9 in tumor immune escape has remained largely unexplored despite the fact that several findings point towards immunosuppressive activity similar to e.g. galectin-1 [79]. First, galectin-9 is well characterized as an eosinophil chemoattractant [80], with gal-9FL being the most potent splice variant for this effect [35]. Eosinophils have predominantly been associated with anti-tumor activity and consequently, the presence of these immune cells is associated to a good prognosis [81]. Interestingly, eosinophilia is often observed in hematological and colon tumors [82–84]. As discussed previously, these tumors frequently display an increased galectin-9 expression compared to normal tissue. At this point, it remains to be determined whether tumor-derived galectin-9 indeed mediates infiltration of eosinophils into the tumor micro-environment and whether loss of galectin-9, as observed in several solid tumor types, indeed allows tumors to escape from an eosinophil-mediated anti-tumor response.

Galectin-9 has also been shown to modulate the differentiation and expansion of other immune cells. For example, galectin-9 administration suppresses the differentiation of T_h17 cells while promoting the differentiation of $CD4^+CD25^+$ regulatory T (T_{reg}) cells in a murine model for collagen-induced arthritis [85]. Interestingly, the galectin-9–Tim-3 axis has been implicated in the immune suppression mediated by T_{regs} [86]. Galectin-9 has also been shown to induce apoptosis in a number of immune cell types like $CD4^+$ type 1 helper T (T_h1) cells [45] and $CD8^+$ cytotoxic T (T_c) cells [87]. At the same time, galectin-9Δ5 promotes the migration of type 2 helper T cells through the extracellular matrix without inducing apoptosis [88]. All this suggests an immunosuppressive activity of galectin-9 that might result in tumor immune escape. This immunosuppressive effect of galectin-9 was indeed proposed as the mechanism by which Epstein–Bar-virus-infected nasopharyngeal carcinoma cells escape immune surveillance [89]. On the other hand, galectin-9 administration was also shown to induce the expansion of dendritic cells resulting in the potentiation of $CD8^+$ or natural killer (NK) cell-mediated anti-tumor immunity in sarcoma and melanoma models respectively [90,91]. Furthermore, the observation that galectin-9 predominantly suppresses immune function seems hard to reconcile with the poor outcome in patients with low galectin-9 expression. Possibly, galectin-9 expression is lost during the course of tumorigenesis, enabling tumor cells to metastasize more easily as soon as multiple other modes of immune escape have developed.

4.4. Angiogenesis

The growth of new blood vessels out of existing capillaries, i.e. angiogenesis, is essential for tumor progression. The vascular cells responsible for this process, i.e. the endothelial cell, express different galectin family members [40] several of which have been shown to regulate tumor angiogenesis, including galectin-8 [11], galectin-3 [12,19,92] and galectin-1 [13,64]. Multiple galectin-9 splice variants have been found to be expressed by endothelial cells [36,40]. Furthermore, we have shown that galectin-9 expression is regulated during endothelial cell activation [40] while others have shown that galectin-9 expression in endothelial cells appears to involve IFNg [57] and HDAC3 [58]. Despite these findings, the exact role of galectin-9 in endothelial cell biology and angiogenesis remains elusive. Although speculative, the protein is possibly involved in attracting eosinophils or expanding dendritic cells, which have been shown to release angiogenic growth factors like VEGF [84,93]. In addition, altered galectin-9 levels on the endothelial cell layer might affect infiltration of anti-tumor immune effector cells, e.g. T_h1 and T_c cells, as well as immune-suppressive immune cells, e.g. eosinophils and T_{regs}, thereby interfering with a proper anti-tumor immune response.

5. Galectin-9 as a potential target for therapeutic intervention in cancer

The emerging paradigm is that galectin-9 acts on multiple levels in tumor biology, similarly to other galectin family members. So far,

galectin-9 has been implicated in tumor cell adhesion and metastasis, immune escape and tumor angiogenesis. From these observations a model emerges in which a decrease in overall intratumoral galectin-9 levels confers resistance to apoptosis and facilitates metastasis while galectin-9 in the tumor vasculature maintains its role in tumor immune escape (Fig. 3). The observation that galectins can exert diverging functions simultaneously and that their expression is often altered in tumors makes these proteins attractive targets for anti-cancer therapy. For example, agents targeting galectin-1 [13,63,94,95] and galectin-3 [96–98] show substantial anti-tumor effects and current data suggest that administration of galectin-9 might be similarly beneficial [99]. In line with this, recombinant stable galectin-9 has been used successfully as therapy in a number of mouse disease models with an overactivated immune system as the underlying cause, like graft versus host disease [86,87], rheumatoid arthritis [85,100] and asthma [46]. These findings have prompted a similar approach for cancers of the immune system. Indeed, in models of leukemia and myeloma treatment with galectin-9 hampered progression by inducing apoptosis [66,101]. In addition, administration of galectin-9 also hampered tumor progression in mouse models of melanoma and colon cancer by blocking metastasis [74]. Unexpectedly, galectin-9 was found to potentiate the immune response in the melanoma model by expanding dendritic cells and potentiating NK cell mediated anti-tumor immunity. A similar effect was reported in a murine model of lung cancer, in which galectin-9 signaling induces the differentiation of macrophages into plasmacytoid dendritic cell-like macrophages [102]. These initial findings are promising and warrant further research into the effect of galectin-9 administration on e.g. metastasis and modulation of immune function in other (solid) cancer types. In addition, it will be of interest to assess the effect of inhibiting rather than potentiating galectin-9 in the tumor vasculature, given that increased endothelial galectin-9 expression might be an important mediator of tumor immune escape. Attempts to find compounds specifically targeting individual galectins have been made [103–105]. Similarly, some compounds are known to target galectin-9, albeit not specific [106]. To our knowledge, no galectin-9 specific inhibitor has been described yet. Apart from interfering with galectin-9 function, the protein itself could also be used to target compounds specifically to e.g. the tumor vasculature, similar as described for galectin-1 [77,78]. Such a strategy could be employed both for therapy and diagnosis of cancer. Finally, no studies have taken the existence of different galectin-9 isoforms into account. Given the opposing effects galectin-9 variants can have on e.g. cell adhesion, it will be important to determine the effects of targeting or administering specific variants in different tumor models.

6. Future perspectives

A role for galectin-9 as a jack of multiple trades in tumor biology is apparent, although many questions remain to be addressed. A better understanding of the molecular mechanisms underlying the various effects observed for galectin-9 is required. A genome-wide screening approach to identify galectin-9 binding partners would be a logical first step in this endeavor. Also, it is now becoming clear that alternative splicing of galectin-9 can have profound effects on the function of the protein. As such, future studies should clearly differentiate between galectin-9 splice variants when exploring galectin-9 function. Current findings identify galectin-9 as a promising target for diagnostic and therapeutic applications. The generation of recombinant galectin-9 isoforms and specific inhibitors of these proteins will allow further exploration of the applicability of galectin-9 in cancer treatment.

References

[1] S.H. Barondes, V. Castronovo, D.N. Cooper, R.D. Cummings, K. Drickamer, T. Feizi, M.A. Gitt, J. Hirabayashi, C. Hughes, K. Kasai, et al., Galectins: a family of animal beta-galactoside-binding lectins, Cell 76 (1994) 597–598.
[2] H. Leffler, S. Carlsson, M. Hedlund, Y. Qian, F. Poirier, Introduction to galectins, Glycoconj. J. 19 (2004) 433–440.
[3] G.A. Rabinovich, M.A. Toscano, S.S. Jackson, G.R. Vasta, Functions of cell surface galectin–glycoprotein lattices, Curr. Opin. Struct. Biol. 17 (2007) 513–520.
[4] G.A. Rabinovich, M.A. Toscano, Turning 'sweet' on immunity: galectin–glycan interactions in immune tolerance and inflammation, Nat. Rev. Immunol. 9 (2009) 338–352.
[5] R.Y. Yang, G.A. Rabinovich, F.T. Liu, Galectins: structure, function and therapeutic potential, Expert Rev. Mol. Med. 10 (2008) e17.
[6] R.C. Hughes, Secretion of the galectin family of mammalian carbohydrate-binding proteins, Biochim. Biophys. Acta 1473 (1999) 172–185.

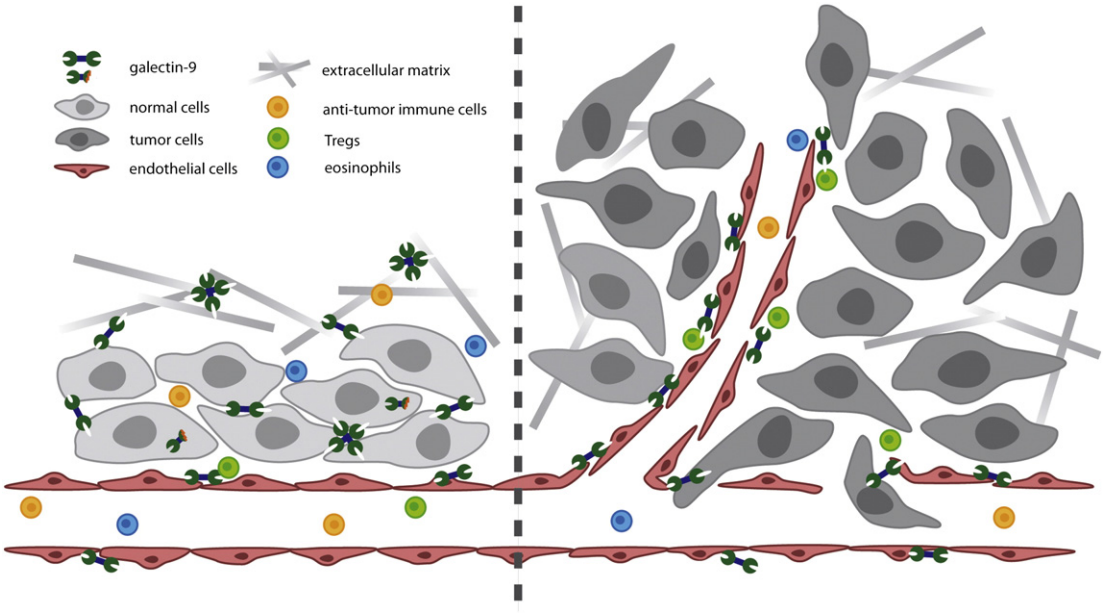

Fig. 3. Established and hypothesized roles of galectin-9 in solid tumor biology. Depicted in the picture is a tissue as it transgresses from a normal physiological state (left side) to solid tumor tissue (right side). This is generally associated with a loss of galectin-9 in the tissue and consequently tissue integrity loss, facilitating metastasis. In addition loss of galectin-9 could confer tumor cells with resistance to apoptosis. Conversely, galectin-9 levels on endothelial cells are increased in the tumor tissue, possibly facilitating metastasis by mediating tumor cell–endothelial cell interactions. Although further experiments are required to test this hypothesis, the high endothelial levels of galectin-9 could contribute to an immune-suppressing boundary separating circulating immune cells from tumor cells, for example by inducing apoptosis of anti-tumor immune effector cells like T_h1 and T_c cells and at the same time facilitating immune-suppression by T_{regs}. In addition, eosinophil attraction near sites of angiogenesis could be maintained.

[7] F.T. Liu, G.A. Rabinovich, Galectins as modulators of tumour progression, Nat. Rev. Cancer 5 (2005) 29–41.
[8] J.L. Wang, R.M. Gray, K.C. Haudek, R.J. Patterson, Nucleocytoplasmic lectins, Biochim. Biophys. Acta 1673 (2004) 75–93.
[9] Y. Imaizumi, M. Sakaguchi, T. Morishita, M. Ito, F. Poirier, K. Sawamoto, H. Okano, Galectin-1 is expressed in early-type neural progenitor cells and down-regulates neurogenesis in the adult hippocampus, Mol. Brain 4 (2011) 7.
[10] I. Comte, Y. Kim, C.C. Young, J.M. van der Harg, P. Hockberger, P.J. Bolam, F. Poirier, F.G. Szele, Galectin-3 maintains cell motility from the subventricular zone to the olfactory bulb, J. Cell Sci. 124 (2011) 2438–2447.
[11] V.M. Cardenas Delgado, L.G. Nugnes, L.L. Colombo, M.F. Troncoso, M.M. Fernandez, E.L. Malchiodi, I. Frahm, D.O. Croci, D. Compagno, G.A. Rabinovich, C. Wolfenstein-Todel, M.T. Elola, Modulation of endothelial cell migration and angiogenesis: a novel function for the "tandem-repeat" lectin galectin-8, FASEB J. 25 (2011) 242–254.
[12] A.I. Markowska, F.T. Liu, N. Panjwani, Galectin-3 is an important mediator of VEGF- and bFGF-mediated angiogenic response, J. Exp. Med. 207 (2010) 1981–1993.
[13] V.L. Thijssen, R. Postel, R.J. Brandwijk, R.P. Dings, I. Nesmelova, S. Satijn, N. Verhofstad, Y. Nakabeppu, L.G. Baum, J. Bakkers, K.H. Mayo, F. Poirier, A.W. Griffioen, Galectin-1 is essential in tumor angiogenesis and is a target for antiangiogenesis therapy, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 15975–15980.
[14] J.M. Ilarregui, D.O. Croci, G.A. Bianco, M.A. Toscano, M. Salatino, M.E. Vermeulen, J.R. Geffner, G.A. Rabinovich, Tolerogenic signals delivered by dendritic cells to T cells through a galectin-1-driven immunoregulatory circuit involving interleukin 27 and interleukin 10, Nat. Immunol. 10 (2009) 981–991.
[15] S.M. Blois, J.M. Ilarregui, M. Tometten, M. Garcia, A.S. Orsal, R. Cordo-Russo, M.A. Toscano, G.A. Bianco, P. Kobelt, B. Handjiski, I. Tirado, U.R. Markert, B.F. Klapp, F. Poirier, J. Szekeres-Bartho, G.A. Rabinovich, P.C. Arck, A pivotal role for galectin-1 in fetomaternal tolerance, Nat. Med. 13 (2007) 1450–1457.
[16] R. Heusschen, N. Freitag, I. Tirado-González, G. Barrientos, P. Moschansky, R. Muñoz-Fernández, E. Leno-Durán, B.F. Klapp, V.L. Thijssen, S.M. Blois, Profiling Lgals9 splice variant expression at the fetal–maternal interface: implications in normal and pathological human pregnancy, Biol. Reprod. 88 (2013) 22.
[17] G. Elad-Sfadia, R. Haklai, E. Balan, Y. Kloog, Galectin-3 augments K-Ras activation and triggers a Ras signal that attenuates ERK but not phosphoinositide 3-kinase activity, J. Biol. Chem. 279 (2004) 34922–34930.
[18] A. Paz, R. Haklai, G. Elad-Sfadia, E. Ballan, Y. Kloog, Galectin-1 binds oncogenic H-Ras to mediate Ras membrane anchorage and cell transformation, Oncogene 20 (2001) 7486–7493.
[19] P. Nangia-Makker, Y. Honjo, R. Sarvis, S. Akahani, V. Hogan, K.J. Pienta, A. Raz, Galectin-3 induces endothelial cell morphogenesis and angiogenesis, Am. J. Pathol. 156 (2000) 899–909.
[20] R.Y. Yang, F.T. Liu, Galectins in cell growth and apoptosis, Cell. Mol. Life Sci. 60 (2003) 267–276.
[21] R.C. Hughes, Galectins as modulators of cell adhesion, Biochimie 83 (2001) 667–676.
[22] I. Kuwabara, H. Sano, F.T. Liu, Functions of galectins in cell adhesion and chemotaxis, Methods Enzymol. 363 (2003) 532–552.
[23] N.L. Perillo, K.E. Pace, J.J. Seilhamer, L.G. Baum, Apoptosis of T cells mediated by galectin-1, Nature 378 (1995) 736–739.
[24] N. Rubinstein, M. Alvarez, N.W. Zwirner, M.A. Toscano, J.M. Ilarregui, A. Bravo, J. Mordoh, L. Fainboim, O.L. Podhajcer, G.A. Rabinovich, Targeted inhibition of galectin-1 gene expression in tumor cells results in heightened T cell-mediated rejection; a potential mechanism of tumor-immune privilege, Cancer Cell 5 (2004) 241–251.
[25] O. Tureci, H. Schmitt, N. Fadle, M. Pfreundschuh, U. Sahin, Molecular definition of a novel human galectin which is immunogenic in patients with Hodgkin's disease, J. Biol. Chem. 272 (1997) 6416–6422.
[26] J. Wada, K. Ota, A. Kumar, E.I. Wallner, Y.S. Kanwar, Developmental regulation, expression, and apoptotic potential of galectin-9, a beta-galactoside binding lectin, J. Clin. Invest. 99 (1997) 2452–2461.
[27] E. Leal-Pinto, W. Tao, J. Rappaport, M. Richardson, B.A. Knorr, R.G. Abramson, Molecular cloning and functional reconstitution of a urate transporter/channel, J. Biol. Chem. 272 (1997) 617–625.
[28] N. Matsushita, N. Nishi, M. Seki, R. Matsumoto, I. Kuwabara, F.T. Liu, Y. Hata, T. Nakamura, M. Hirashima, Requirement of divalent galactoside-binding activity of ecalectin/galectin-9 for eosinophil chemoattraction, J. Biol. Chem. 275 (2000) 8355–8360.
[29] K. Kimura, A. Wakamatsu, Y. Suzuki, T. Ota, T. Nishikawa, R. Yamashita, J. Yamamoto, M. Sekine, K. Tsuritani, H. Wakaguri, S. Ishii, T. Sugiyama, K. Saito, Y. Isono, R. Irie, N. Kushida, T. Yoneyama, R. Otsuka, K. Kanda, T. Yokoi, H. Kondo, M. Wagatsuma, K. Murakawa, S. Ishida, T. Ishibashi, A. Takahashi-Fujii, T. Tanase, K. Nagai, H. Kikuchi, K. Nakai, T. Isogai, S. Sugano, Diversification of transcriptional modulation: large-scale identification and characterization of putative alternative promoters of human genes, Genome Res. 16 (2006) 55–65.
[30] J.H. Oh, J.O. Yang, Y. Hahn, M.R. Kim, S.S. Byun, Y.J. Jeon, J.M. Kim, K.S. Song, S.M. Noh, S. Kim, H.S. Yoo, Y.S. Kim, N.S. Kim, Transcriptome analysis of human gastric cancer, Mamm. Genome 16 (2005) 942–954.
[31] R.L. Strausberg, E.A. Feingold, L.H. Grouse, J.G. Derge, R.D. Klausner, F.S. Collins, L. Wagner, C.M. Shenmen, G.D. Schuler, S.F. Altschul, B. Zeeberg, K.H. Buetow, C.F. Schaefer, N.K. Bhat, R.F. Hopkins, H. Jordan, T. Moore, S.I. Max, J. Wang, F. Hsieh, L. Diatchenko, K. Marusina, A.A. Farmer, G.M. Rubin, L. Hong, M. Stapleton, M.B. Soares, M.F. Bonaldo, T.L. Casavant, T.E. Scheetz, M.J. Brownstein, T.B. Usdin, S. Toshiyuki, P. Carninci, C. Prange, S.S. Raha, N.A. Loquellano, G.J. Peters, R.D. Abramson, S.J. Mullahy, S.A. Bosak, P.J. McEwan, K.J. McKernan, J.A. Malek, P.H. Gunaratne, S. Richards, K.C. Worley, S. Hale, A.M. Garcia, L.J. Gay, S.W. Hulyk, D.K. Villalon, D.M. Muzny, E.J. Sodergren, X. Lu, R.A. Gibbs, J. Fahey, E. Helton, M. Ketteman, A. Madan, S. Rodrigues, A. Sanchez, M. Whiting, A. Madan, A.C. Young, Y. Shevchenko, G.G. Bouffard, R.W. Blakesley, J.W. Touchman, E.D. Green, M.C. Dickson, A.C. Rodriguez, J. Grimwood, J. Schmutz, R.M. Myers, Y.S. Butterfield, M.I. Krzywinski, U. Skalska, D.E. Smailus, A. Schnerch, J.E. Schein, S.J. Jones, M.A. Marra, Mammalian Gene Collection Program Team, Generation and initial analysis of more than 15,000 full-length human and mouse cDNA sequences, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 16899–16903.
[32] N. Bidon, F. Brichory, S. Hanash, P. Bourguet, L. Dazord, J.P. Le Pennec, Two messenger RNAs and five isoforms for Po66-CBP, a galectin-8 homolog in a human lung carcinoma cell line, Gene 274 (2001) 253–262.
[33] N. Bidon, F. Brichory, P. Bourguet, J.P. Le Pennec, L. Dazord, Galectin-8: a complex sub-family of galectins (review), Int. J. Mol. Med. 8 (2001) 245–250.
[34] H. Ahmed, P.P. Banerjee, G.R. Vasta, Differential expression of galectins in normal, benign and malignant prostate epithelial cells: silencing of galectin-3 expression in prostate cancer by its promoter methylation, Biochem. Biophys. Res. Commun. 358 (2007) 241–246.
[35] S. Chabot, Y. Kashio, M. Seki, Y. Shirato, K. Nakamura, N. Nishi, T. Nakamura, R. Matsumoto, M. Hirashima, Regulation of galectin-9 expression and release in Jurkat T cell line cells, Glycobiology 12 (2002) 111–118.
[36] F. Spitzenberger, J. Graessler, H.E. Schroeder, Molecular and functional characterization of galectin 9 mRNA isoforms in porcine and human cells and tissues, Biochimie 83 (2001) 851–862.
[37] S. Bi, L.A. Earl, L. Jacobs, L.G. Baum, Structural features of galectin-9 and galectin-1 that determine distinct T cell death pathways, J. Biol. Chem. 283 (2008) 12248–12258.
[38] L.A. Earl, S. Bi, L.G. Baum, Galectin multimerization and lattice formation are regulated by linker region structure, Glycobiology 21 (2011) 6–12.
[39] M. Hirashima, Y. Kashio, N. Nishi, A. Yamauchi, T.A. Imaizumi, T. Kageshita, N. Saita, T. Nakamura, Galectin-9 in physiological and pathological conditions, Glycoconj. J. 19 (2004) 593–600.
[40] V.L. Thijssen, S. Hulsmans, A.W. Griffioen, The galectin profile of the endothelium; altered expression and localization in activated and tumor endothelial cells, Am. J. Pathol. 172 (2008) 545–553.
[41] M.S. Lipkowitz, E. Leal-Pinto, B.E. Cohen, R.G. Abramson, Galectin 9 is the sugar-regulated urate transporter/channel UAT, Glycoconj. J. 19 (2004) 491–498.
[42] H. Asakura, Y. Kashio, K. Nakamura, M. Seki, S. Dai, Y. Shirato, M.J. Abedin, N. Yoshida, N. Nishi, T. Imaizumi, N. Saita, Y. Toyama, H. Takashima, T. Nakamura, M. Ohkawa, M. Hirashima, Selective eosinophil adhesion to fibroblast via IFN-gamma-induced galectin-9, J. Immunol. 169 (2002) 5912–5918.
[43] H. Yoshida, M. Teraoka, N. Nishi, S. Nakakita, T. Nakamura, M. Hirashima, S. Kamitori, X-ray structures of human galectin-9 C-terminal domain in complexes with a biantennary oligosaccharide and sialyllactose, J. Biol. Chem. 285 (2010) 36969–36976.
[44] M. Nagae, N. Nishi, T. Murata, T. Usui, T. Nakamura, S. Wakatsuki, R. Kato, Structural analysis of the recognition mechanism of poly-N-acetyllactosamine by the human galectin-9 N-terminal carbohydrate recognition domain, Glycobiology 19 (2009) 112–117.
[45] C. Zhu, A.C. Anderson, A. Schubart, H. Xiong, J. Imitola, S.J. Khoury, X.X. Zheng, T.B. Strom, V.K. Kuchroo, The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity, Nat. Immunol. 6 (2005) 1245–1252.
[46] S. Katoh, N. Ishii, A. Nobumoto, K. Takeshita, S.Y. Dai, R. Shinonaga, T. Niki, N. Nishi, A. Tominaga, A. Yamauchi, M. Hirashima, Galectin-9 inhibits CD44–hyaluronan interaction and suppresses a murine model of allergic asthma, Am. J. Respir. Crit. Care Med. 176 (2007) 27–35.
[47] A. Matsuura, J. Tsukada, T. Mizobe, T. Higashi, F. Mouri, R. Tanikawa, A. Yamauchi, M. Hirashima, Y. Tanaka, Intracellular galectin-9 activates inflammatory cytokines in monocytes, Genes Cells 14 (2009) 511–521.
[48] H. Lahm, S. Andre, A. Hoeflich, J.R. Fischer, B. Sordat, H. Kaltner, E. Wolf, H.J. Gabius, 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 (2001) 375–386.
[49] A. Kasamatsu, K. Uzawa, D. Nakashima, H. Koike, M. Shiiba, H. Bukawa, H. Yokoe, H. Tanzawa, Galectin-9 as a regulator of cellular adhesion in human oral squamous cell carcinoma cell lines, Int. J. Mol. Med. 16 (2005) 269–273.
[50] A. Irie, A. Yamauchi, K. Kontani, M. Kihara, D. Liu, Y. Shirato, M. Seki, N. Nishi, T. Nakamura, H. Yokomise, M. Hirashima, Galectin-9 as a prognostic factor with antimetastatic potential in breast cancer, Clin. Cancer Res. 11 (2005) 2962–2968.
[51] T. Kageshita, Y. Kashio, A. Yamauchi, M. Seki, M.J. Abedin, N. Nishi, H. Shoji, T. Nakamura, T. Ono, M. Hirashima, Possible role of galectin-9 in cell aggregation and apoptosis of human melanoma cell lines and its clinical significance, Int. J. Cancer 99 (2002) 809–816.
[52] A. Danguy, I. Camby, R. Kiss, Galectins and cancer, Biochim. Biophys. Acta 1572 (2002) 285–293.
[53] F. van den Brule, S. Califice, V. Castronovo, Expression of galectins in cancer: a critical review, Glycoconj. J. 19 (2004) 537–542.
[54] M. Liang, M. Ueno, S. Oomizu, T. Arikawa, R. Shinonaga, S. Zhang, A. Yamauchi, M. Hirashima, Galectin-9 expression links to malignant potential of cervical squamous cell carcinoma, J. Cancer Res. Clin. Oncol. 134 (2008) 899–907.
[55] Z.Y. Zhang, J.H. Dong, Y.W. Chen, X.Q. Wang, C.H. Li, J. Wang, G.Q. Wang, H.L. Li, X.D. Wang, Galectin-9 acts as a prognostic factor with antimetastatic potential in hepatocellular carcinoma, Asian Pac. J. Cancer Prev. 13 (2012) 2503–2509.
[56] H. Lahm, A. Hoeflich, S. Andre, B. Sordat, H. Kaltner, E. Wolf, H.J. Gabius, Gene expression of galectin-9/ecalectin, a potent eosinophil chemoattractant, and/or the insertional isoform in human colorectal carcinoma cell lines and detection of frame-shift mutations for protein sequence truncations in the second functional lectin domain, Int. J. Oncol. 17 (2000) 519–524.

[57] T. Imaizumi, M. Kumagai, N. Sasaki, H. Kurotaki, F. Mori, M. Seki, N. Nishi, K. Fujimoto, K. Tanji, T. Shibata, W. Tamo, T. Matsumiya, H. Yoshida, X.-F. Cui, S. Takanashi, K. Hanada, K. Okumura, S. Yagihashi, K. Wakabayashi, T. Nakamura, M. Hirashima, K. Satoh, Interferon-{gamma} stimulates the expression of galectin-9 in cultured human endothelial cells, J. Leukoc. Biol. 72 (2002) 486–491.
[58] S. Alam, H. Li, A. Margariti, D. Martin, A. Zampetaki, O. Habi, G. Cockerill, Y. Hu, Q. Xu, L. Zeng, Galectin-9 protein expression in endothelial cells is positively regulated by histone deacetylase 3, J. Biol. Chem. 286 (2011) 44211–44217.
[59] H. Yoshida, T. Imaizumi, M. Kumagai, K. Kimura, C. Satoh, N. Hanada, K. Fujimoto, N. Nishi, K. Tanji, T. Matsumiya, F. Mori, X.F. Cui, W. Tamo, T. Shibata, S. Takanashi, K. Okumura, T. Nakamura, K. Wakabayashi, M. Hirashima, Y. Sato, K. Satoh, Interleukin-1beta stimulates galectin-9 expression in human astrocytes, Neuroreport 12 (2001) 3755–3758.
[60] N. Saita, E. Goto, T. Yamamoto, I. Cho, K. Tsumori, H. Kohrogi, K. Maruo, T. Ono, M. Takeya, Y. Kashio, K. Nakamura, M. Hirashima, Association of galectin-9 with eosinophil apoptosis, Int. Arch. Allergy Immunol. 128 (2002) 42–50.
[61] F. van den Brule, S. Califice, F. Garnier, P.L. Fernandez, A. Berchuck, V. Castronovo, 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 (2003) 377–386.
[62] M.V. Espelt, D.O. Croci, M.L. Bacigalupo, P. Carabias, M. Manzi, M.T. Elola, M.C. Muñoz, F.P. Dominici, C. Wolfenstein-Todel, G.A. Rabinovich, M.F. Troncoso, Novel roles of galectin-1 in hepatocellular carcinoma cell adhesion, polarization, and in vivo tumor growth, Hepatology 53 (2011) 2097–2106.
[63] K. Ito, S.J. Ralph, Inhibiting galectin-1 reduces murine lung metastasis with increased CD4($^+$) and CD8 ($^+$) T cells and reduced cancer cell adherence, Clin. Exp. Metastasis 29 (2012) 561–572.
[64] V.L. Thijssen, B. Barkan, H. Shoji, I.M. Aries, V. Mathieu, L. Deltour, T.M. Hackeng, R. Kiss, Y. Kloog, F. Poirier, A.W. Griffioen, Tumor cells secrete galectin-1 to enhance endothelial cell activity, Cancer Res. 70 (2010) 6216–6224.
[65] F.T. Liu, R.Y. Yang, J. Saegusa, H.Y. Chen, D.K. Hsu, Galectins in regulation of apoptosis, Adv. Exp. Med. Biol. 705 (2011) 431–442.
[66] T. Kobayashi, J. Kuroda, E. Ashihara, S. Oomizu, Y. Terui, A. Taniyama, S. Adachi, T. Takagi, M. Yamamoto, N. Sasaki, S. Horiike, K. Hatake, A. Yamauchi, M. Hirashima, M. Taniwaki, Galectin-9 exhibits anti-myeloma activity through JNK and p38 MAP kinase pathways, Leukemia 24 (2010) 843–850.
[67] Y. Kashio, K. Nakamura, M.J. Abedin, M. Seki, N. Nishi, N. Yoshida, T. Nakamura, M. Hirashima, Galectin-9 induces apoptosis through the calcium–calpain–caspase-1 pathway, J. Immunol. 170 (2003) 3631–3636.
[68] L.H. Lu, R. Nakagawa, Y. Kashio, A. Ito, H. Shoji, N. Nishi, M. Hirashima, A. Yamauchi, T. Nakamura, Characterization of galectin-9-induced death of Jurkat T cells, J. Biochem. 141 (2007) 157–172.
[69] E.W. Su, S. Bi, L.P. Kane, Galectin-9 regulates T helper cell function independently of Tim-3, Glycobiology 21 (2011) 1258–1265.
[70] V. Vas, R. Fajka-Boja, G. Ion, V. Dudics, E. Monostori, F. Uher, Biphasic effect of recombinant galectin-1 on the growth and death of early hematopoietic cells, Stem Cells 23 (2005) 279–287.
[71] S. Califice, V. Castronovo, M. Bracke, F. van den Brule, Dual activities of galectin-3 in human prostate cancer: tumor suppression of nuclear galectin-3 vs tumor promotion of cytoplasmic galectin-3, Oncogene 23 (2004) 7527–7536.
[72] K.S. Kim, K.W. Kang, Y.B. Seu, S.H. Baek, J.R. Kim, Interferon-gamma induces cellular senescence through p53-dependent DNA damage signaling in human endothelial cells, Mech. Ageing Dev. 130 (2009) 179–188.
[73] F. Zhang, M. Zheng, Y. Qu, J. Li, J. Ji, B. Feng, A. Lu, J. Li, M. Wang, B. Liu, Different roles of galectin-9 isoforms in modulating E-selectin expression and adhesion function in LoVo colon carcinoma cells, Mol. Biol. Rep. 36 (2009) 823–830.
[74] A. Nobumoto, K. Nagahara, S. Oomizu, S. Katoh, N. Nishi, K. Takeshita, T. Niki, A. Tominaga, A. Yamauchi, M. Hirashima, Galectin-9 suppresses tumor metastasis by blocking adhesion to endothelium and extracellular matrices, Glycobiology 18 (2008) 735–744.
[75] J. Friedrichs, A. Manninen, D.J. Muller, J. Helenius, Galectin-3 regulates integrin alpha2beta1-mediated adhesion to collagen-I and -IV, J. Biol. Chem. 283 (2008) 32264–32272.
[76] H. Sanchez-Ruderisch, K.M. Detjen, M. Welzel, S. Andre, C. Fischer, H.J. Gabius, S. Rosewicz, Galectin-1 sensitizes carcinoma cells to anoikis via the fibronectin receptor alpha(5)beta(1)-integrin, Cell Death Differ. 18 (2011) 806–816.
[77] E. Kluza, I. Jacobs, S.J. Hectors, K.H. Mayo, A.W. Griffioen, G.J. Strijkers, K. Nicolay, Dual-targeting of αvβ3 and galectin-1 improves the specificity of paramagnetic/fluorescent liposomes to tumor endothelium in vivo, J. Control. Release 158 (2012) 207–214.
[78] E. Kluza, D.W. van der Schaft, P.A. Hautvast, W.J. Mulder, K.H. Mayo, A.W. Griffioen, G.J. Strijkers, K. Nicolay, Synergistic targeting of alphavbeta3 integrin and galectin-1 with heteromultivalent paramagnetic liposomes for combined MR imaging and treatment of angiogenesis, Nano Lett. 10 (2010) 52–58.
[79] G.A. Rabinovich, D.O. Croci, Regulatory circuits mediated by lectin–glycan interactions in autoimmunity and cancer, Immunity 36 (2012) 322–335.
[80] M. Hirashima, Ecalectin/galectin-9, a novel eosinophil chemoattractant: its function and production, Int. Arch. Allergy Immunol. 122 (Suppl. 1) (2000) 6–9.
[81] S. Gatault, F. Legrand, M. Delbeke, S. Loiseau, M. Capron, Involvement of eosinophils in the anti-tumor response, Cancer Immunol. Immunother. 61 (2012) 1527–1534.
[82] J. Wedemeyer, K. Vosskuhl, Role of gastrointestinal eosinophils in inflammatory bowel disease and intestinal tumours, Best Pract. Res. Clin. Gastroenterol. 22 (2008) 537–549.
[83] M. Samoszuk, Eosinophils and human cancer, Histol. Histopathol. 12 (1997) 807–812.
[84] A. Munitz, F. Levi-Schaffer, Eosinophils: 'new' roles for 'old' cells, Allergy 59 (2004) 268–275.
[85] M. Seki, S. Oomizu, K.M. Sakata, A. Sakata, T. Arikawa, K. Watanabe, K. Ito, K. Takeshita, T. Niki, N. Saita, N. Nishi, A. Yamauchi, S. Katoh, A. Matsukawa, V. Kuchroo, M. Hirashima, Galectin-9 suppresses the generation of Th17, promotes the induction of regulatory T cells, and regulates experimental autoimmune arthritis, Clin. Immunol. 127 (2008) 78–88.
[86] F. Wang, W. He, J. Yuan, K. Wu, H. Zhou, W. Zhang, Z.K. Chen, Activation of Tim-3–galectin-9 pathway improves survival of fully allogeneic skin grafts, Transpl. Immunol. 19 (2008) 12–19.
[87] F. Wang, W. He, H. Zhou, J. Yuan, K. Wu, L. Xu, Z.K. Chen, The Tim-3 ligand galectin-9 negatively regulates CD8$^+$ alloreactive T cell and prolongs survival of skin graft, Cell. Immunol. 250 (2007) 68–74.
[88] S. Bi, P.W. Hong, B. Lee, L.G. Baum, Galectin-9 binding to cell surface protein disulfide isomerase regulates the redox environment to enhance T-cell migration and HIV entry, Proc. Natl. Acad. Sci. 108 (2011) 10650–10655.
[89] C. Keryer-Bibens, C. Pioche-Durieu, C. Villemant, S. Souquère, N. Nishi, M. Hirashima, J. Middeldorp, P. Busson, Exosomes released by EBV-infected nasopharyngeal carcinoma cells convey the viral latent membrane protein 1 and the immunomodulatory protein galectin 9, BMC Cancer 6 (2006) 283.
[90] K. Nagahara, T. Arikawa, S. Oomizu, K. Kontani, A. Nobumoto, H. Tateno, K. Watanabe, T. Niki, S. Katoh, M. Miyake, S. Nagahata, J. Hirabayashi, V.K. Kuchroo, A. Yamauchi, M. Hirashima, Galectin-9 increases Tim-3$^+$ dendritic cells and CD8$^+$ T cells and enhances antitumor immunity via galectin-9–Tim-3 interactions, J. Immunol. 181 (2008) 7660–7669.
[91] A. Nobumoto, S. Oomizu, T. Arikawa, S. Katoh, K. Nagahara, M. Miyake, N. Nishi, K. Takeshita, T. Niki, A. Yamauchi, M. Hirashima, Galectin-9 expands unique macrophages exhibiting plasmacytoid dendritic cell-like phenotypes that activate NK cells in tumor-bearing mice, Clin. Immunol. 130 (2009) 322–330.
[92] P. Nangia-Makker, Y. Wang, T. Raz, L. Tait, V. Balan, V. Hogan, A. Raz, Cleavage of galectin-3 by matrix metalloproteases induces angiogenesis in breast cancer, Int. J. Cancer 127 (2010) 2530–2541.
[93] O. Fainaru, N. Almog, C.W. Yung, K. Nakai, M. Montoya-Zavala, A. Abdollahi, R. D'Amato, D.E. Ingber, Tumor growth and angiogenesis are dependent on the presence of immature dendritic cells, FASEB J. 24 (2010) 1411–1418.
[94] R.J. Brandwijk, R.P. Dings, E. van der Linden, K.H. Mayo, V.L. Thijssen, A.W. Griffioen, Anti-angiogenesis and anti-tumor activity of recombinant anginex, Biochem. Biophys. Res. Commun. 349 (2006) 1073–1078.
[95] K. Ito, S.A. Scott, S. Cutler, L.F. Dong, J. Neuzil, H. Blanchard, S.J. Ralph, Thiodigalactoside inhibits murine cancers by concurrently blocking effects of galectin-1 on immune dysregulation, angiogenesis and protection against oxidative stress, Angiogenesis 14 (2011) 293–307.
[96] O.V. Glinskii, S. Sud, V.V. Mossine, T.P. Mawhinney, D.C. Anthony, G.V. Glinsky, K.J. Pienta, V.V. Glinsky, Inhibition of prostate cancer bone metastasis by synthetic TF antigen mimic/galectin-3 inhibitor lactulose-l-leucine, Neoplasia 14 (2012) 65–73.
[97] H.Y. Liu, Z.L. Huang, G.H. Yang, W.Q. Lu, N.R. Yu, Inhibitory effect of modified citrus pectin on liver metastases in a mouse colon cancer model, World J. Gastroenterol. 14 (2008) 7386–7391.
[98] P. Nangia-Makker, V. Hogan, Y. Honjo, S. Baccarini, L. Tait, R. Bresalier, A. Raz, Inhibition of human cancer cell growth and metastasis in nude mice by oral intake of modified citrus pectin, J. Natl. Cancer Inst. 94 (2002) 1854–1862.
[99] V.R. Wiersma, M. de Bruyn, W. Helfrich, E. Bremer, Therapeutic potential of Galectin-9 in human disease, Med. Res. Rev. 33 (Suppl 1) (2011) E102–E126.
[100] M. Seki, K.M. Sakata, S. Oomizu, T. Arikawa, A. Sakata, M. Ueno, A. Nobumoto, T. Niki, N. Saita, K. Ito, S.Y. Dai, S. Katoh, N. Nishi, M. Tsukano, K. Ishikawa, A. Yamauchi, V. Kuchroo, M. Hirashima, Beneficial effect of galectin 9 on rheumatoid arthritis by induction of apoptosis of synovial fibroblasts, Arthritis Rheum. 56 (2007) 3968–3976.
[101] J. Kuroda, M. Yamamoto, H. Nagoshi, T. Kobayashi, N. Sasaki, Y. Shimura, S. Horiike, S. Kimura, A. Yamauchi, M. Hirashima, M. Taniwaki, Targeting activating transcription factor 3 by galectin-9 induces apoptosis and overcomes various types of treatment resistance in chronic myelogenous leukemia, Mol. Cancer Res. 8 (2010) 994–1001.
[102] T. Kadowaki, T. Arikawa, R. Shinonaga, S. Oomizu, H. Inagawa, G. Soma, T. Niki, M. Hirashima, Galectin-9 signaling prolongs survival in murine lung-cancer by inducing macrophages to differentiate into plasmacytoid dendritic cell-like macrophages, Clin. Immunol. 142 (2012) 296–307.
[103] P.M. Collins, C.T. Oberg, H. Leffler, U.J. Nilsson, H. Blanchard, Taloside inhibitors of galectin-1 and galectin-3, Chem. Biol. Drug Des. 79 (2012) 339–346.
[104] K.A. Stannard, P.M. Collins, K. Ito, E.M. Sullivan, S.A. Scott, E. Gabutero, I. Darren Grice, P. Low, U.J. Nilsson, H. Leffler, H. Blanchard, S.J. Ralph, Galectin inhibitory disaccharides promote tumour immunity in a breast cancer model, Cancer Lett. 299 (2010) 95–110.
[105] L. Ingrassia, I. Camby, F. Lefranc, V. Mathieu, P. Nshimyumukiza, F. Darro, R. Kiss, Anti-galectin compounds as potential anti-cancer drugs, Curr. Med. Chem. 13 (2006) 3513–3527.
[106] E. Salomonsson, V.L. Thijssen, A.W. Griffioen, U.J. Nilsson, H. Leffler, The anti-angiogenic peptide anginex greatly enhances galectin-1 binding affinity for glycoproteins, J. Biol. Chem. 286 (2011) 13801–13804.
[107] D. Solís, M.J. Maté, M. Lohr, J.P. Ribeiro, L. López-Merino, S. André, E. Buzamet, F.J. Cañada, H. Kaltner, M. Lensch, F.M. Ruiz, G. Haroske, U. Wollina, M. Kloor, J. Kopitz, J.L. Sáiz, M. Menéndez, J. Jiménez-Barbero, A. Romero, H.J. Gabius, N-domain of human adhesion/growth-regulatory galectin-9: preference for distinct conformers and non-sialylated N-glycans and detection of ligand-induced structural changes in crystal and solution, Int. J. Biochem. Cell Biol. 42 (2010) 1019–1029.
[108] R. Tanikawa, T. Tanikawa, M. Hirashima, A. Yamauchi, Y. Tanaka, Galectin-9 induces osteoblast differentiation through the CD44/Smad signaling pathway, Biochem. Biophys. Res. Commun. 394 (2010) 317–322.
[109] C. Pioche-Durieu, C. Keryer, S. Souquere, J. Bosq, W. Faigle, D. Loew, M. Hirashima, N. Nishi, J. Middeldorp, P. Busson, In nasopharyngeal carcinoma cells, Epstein–

Barr virus LMP1 interacts with galectin 9 in membrane raft elements resistant to simvastatin, J. Virol. 79 (2005) 13326–13337.
[110] I. Pelletier, T. Hashidate, T. Urashima, N. Nishi, T. Nakamura, M. Futai, Y. Arata, K. Kasai, M. Hirashima, J. Hirabayashi, S. Sato, Specific recognition of Leishmania major poly-beta-galactosyl epitopes by galectin-9: possible implication of galectin-9 in interaction between L. major and host cells, J. Biol. Chem. 278 (2003) 22223–22230.
[111] N. Nishi, A. Itoh, H. Shoji, H. Miyanaka, T. Nakamura, Galectin-8 and galectin-9 are novel substrates for thrombin, Glycobiology 16 (2006) 15C–20C.
[112] N. Miyanishi, N. Nishi, H. Abe, Y. Kashio, R. Shinonaga, S. Nakakita, W. Sumiyoshi, A. Yamauchi, T. Nakamura, M. Hirashima, J. Hirabayashi, Carbohydrate-recognition domains of galectin-9 are involved in intermolecular interaction with galectin-9 itself and other members of the galectin family, Glycobiology 17 (2007) 423–432.
[113] T. Niki, S. Tsutsui, S. Hirose, S. Aradono, Y. Sugimoto, K. Takeshita, N. Nishi, M. Hirashima, Galectin-9 is a high affinity IgE-binding lectin with anti-allergic effect by blocking IgE–antigen complex formation, J. Biol. Chem. 284 (2009) 32344–32352.