

blood

2007 110: 2819-2827
Prepublished online June 25, 2007;
doi:10.1182/blood-2007-03-077792

Galectins in the tumor endothelium: opportunities for combined cancer therapy

Victor L. J. L. Thijssen, Françoise Poirier, Linda G. Baum and Arjan W. Griffioen

Updated information and services can be found at:

<http://bloodjournal.hematologylibrary.org/content/110/8/2819.full.html>

Articles on similar topics can be found in the following Blood collections

[Cell Adhesion and Motility](#) (790 articles)

[Hemostasis, Thrombosis, and Vascular Biology](#) (2497 articles)

[Neoplasia](#) (4217 articles)

[Signal Transduction](#) (1930 articles)

Information about reproducing this article in parts or in its entirety may be found online at:

http://bloodjournal.hematologylibrary.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:

<http://bloodjournal.hematologylibrary.org/site/misc/rights.xhtml#reprints>

Information about subscriptions and ASH membership may be found online at:

<http://bloodjournal.hematologylibrary.org/site/subscriptions/index.xhtml>

Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.

Copyright 2011 by The American Society of Hematology; all rights reserved.



Galectins in the tumor endothelium: opportunities for combined cancer therapy

Victor L. J. L. Thijssen¹, Françoise Poirier², Linda G. Baum³, and Arjan W. Griffioen¹

¹Angiogenesis Laboratory, Research Institute for Growth and Development (GROW), Department of Pathology, University Maastricht and Academic Hospital Maastricht, the Netherlands; ²Institute Jacques Monod, Unité Mixte de Recherche, Centre National de la Recherche Scientifique (UMR CNRS) 7592, Paris, France; ³Department of Pathology and Laboratory Medicine, University of California School of Medicine, Los Angeles, CA

Galectins are emerging as a family of proteins that play an important role in several steps of tumorigenesis. Evidence is accumulating that galectins are expressed by the tumor endothelium, where they contribute to different steps of tumor progression such as immune escape and metastasis. Recent

studies have identified an important role for galectins in tumor angiogenesis. Moreover, it has been shown that galectins in the endothelium can be targeted for therapeutic applications. This opens a window of opportunity for the development of tumor-type independent treatment strategies. This re-

view focuses on the expression of galectins in the tumor endothelium, their contribution to tumor progression, and their application in tumor-type independent cancer therapy. (Blood. 2007;110:2819-2827)

© 2007 by The American Society of Hematology

Introduction

Cancer cells induce the growth of new blood vessels to grow beyond the size of a few cubic millimeters. This neovascularization, or angiogenesis, ensures proper blood supply and facilitates tumor metastasis (Figure 1). The dependence on neovascularization has made tumor angiogenesis an attractive target for therapeutic applications.¹

It is thought that angiogenesis in different tumor types follows similar steps, allowing tumor-independent treatment strategies.² Furthermore, the cells that play a pivotal role in angiogenesis (ie, the endothelial cells [ECs]) are particularly suitable targets for treatment. They line the luminal side of vessels and are in direct contact with blood, making them easily accessible. Furthermore, disrupting the tumor vasculature by killing the ECs results in massive loss of tumor cells.³ Finally, ECs are expected to be less susceptible to development of drug-induced resistance.

Effective EC-targeted therapy depends on the availability of molecules that can distinguish the tumor ECs from ECs in normal tissues.⁴ Currently, a limited number of such molecules has been identified, including integrins $\alpha_5\beta_1$, $\alpha_v\beta_3$, and $\alpha_v\beta_5$; oncofetal fibronectin; ROBO4; CD36; and CD13.^{5,6} We recently described galectin-1 as a protein that is overexpressed on activated tumor ECs in vivo. We showed that tumor vessels hardly develop in mutant galectin-1 knockout mice.⁷ Evidence is accumulating that other galectin family members are also expressed by the endothelium, and that they contribute to different steps of tumor progression such as immune escape and metastasis. Moreover, it has been shown that galectins in the endothelium can be targeted for therapeutic applications.⁷⁻¹⁰ This opens opportunities for the development of tumor-type independent treatment strategies and justifies a review on the role of galectins in the tumor endothelium.

galectins have been identified that all share a conserved carbohydrate recognition domain (CRD) of approximately 130 amino acids.¹¹ Based on their structure and the number of CRDs, galectins can be subdivided into 3 groups (Table 1): (1) prototype galectins, consisting of a single CRD (galectins-1, -2, -5, -7, -10, -11, -13, and -14); (2) tandem repeat galectins, consisting of 2 different but homologous CRDs, connected by a linker region (galectins-4, -6, -8, -9, and -12); and (3) chimera galectins, which have a tail of short tandem repeats fused to a single CRD (galectin-3). They can be secreted by nonclassical pathways¹² and—depending on the cell type or differentiation state—they are found in the nucleus and the cytoplasm, on the cell surface, and in the extracellular matrix.^{13,14}

Members of the galectin family are found in vertebrates, invertebrates, and protists. Galectin-related sequences have even been found in plants and viruses. This degree of conservation suggests an important role in basic cellular mechanisms.^{11,15} Indeed, galectins have been shown to be involved in cell interactions, proliferation, migration, apoptosis, and mRNA splicing (Figure 2).^{13,16-18} Galectins are also associated with different pathologies, and most importantly with cancer.¹⁹ Recent reviews have summarized the current knowledge on the expression of different galectins (eg, galectin-1, galectin-3,²⁰ galectin-4,²¹ and galectin-8)⁸ in a variety of tumor types. They all conclude that galectins in tumor cells might provide targets for novel anticancer therapies. This is not surprising, since many functions of galectins, notably control of cell interactions/adhesion, effects on cell proliferation/apoptosis, and immunosuppressive properties, have been proposed to account for their role in tumor progression.¹⁹

The galectin protein family

Galectins are a family of carbohydrate-binding proteins with a high affinity for β -galactosides on the cell surface and extracellular glycoproteins as well as on glycolipids. So far, 15 mammalian

Galectin expression in the endothelium

The contribution of endothelial galectins to tumor progression has received relatively little attention. This is surprising because ECs have been found to express several galectins, and the endothelium is recognized as a good target for tumor-type independent therapy.

Submitted March 5, 2007; accepted June 21, 2007. Prepublished online as *Blood* First Edition paper, June 25, 2007; DOI 10.1182/blood-2007-03-077792.

© 2007 by The American Society of Hematology

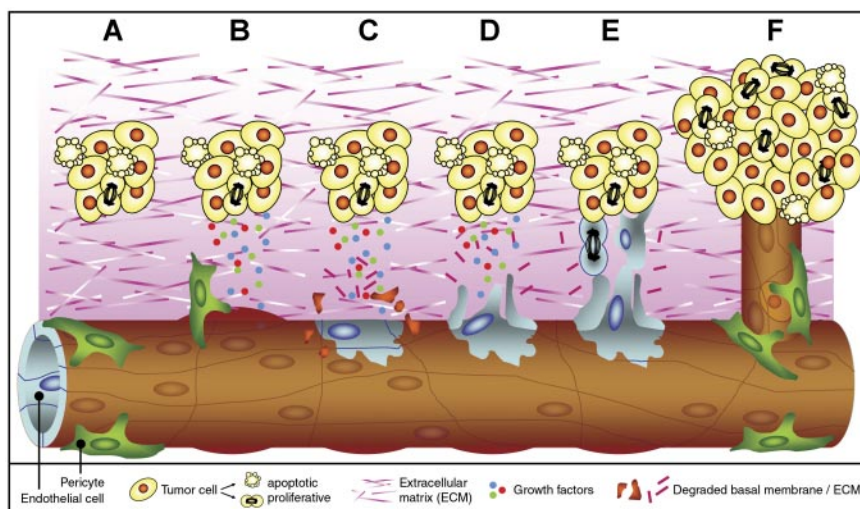


Figure 1. Tumor angiogenesis. Tumor angiogenesis is a multistep process that occurs in almost all tumors and that is mediated by endothelial cells (ECs). When a cell has acquired genetic alterations that allow unlimited growth and escape from apoptosis, a small tumor is formed (A). As soon as the tumor volume has reached a few cubic millimeters, oxygen and nutrient supply are insufficient, and the tumor cells undergo an angiogenesis switch. This results in the production and release of growth factors into the surrounding tissue. The secreted growth factors bind to receptors on ECs in nearby vessels. Pericytes that stabilize the vessel detach, and vessel dilation occurs (B). In addition, the activated ECs start to produce proteases (not shown) that degrade the basal membrane and the extracellular matrix (C). Subsequently, the ECs start to migrate (D) and proliferate (E) into the growth factor gradient, forming new vascular structures. Finally, matrix proteins are deposited, and the new vessel is stabilized by pericytes to form a functional and mature blood vessel. The tumor cells can continue to grow, and metastasis formation is facilitated since the tumor cells now have easy access to the circulation (F).

Galectin-1

Galectin-1 is the first galectin described in literature (for a recent review, see Camby et al²²). It exists as a noncovalent homodimer of 2 14.5-kDa CRDs. It can be found intracellularly, extracellularly, and at the cell surface of different cell types. Each dimer can bind 2 sugar residues to mediate intramolecular and intermolecular interactions. Furthermore, galectin-1 can be involved in carbohydrate-independent protein-protein interactions, which further contributes to the variety of its biological functions.^{23,24} Several galectin-1-binding partners have been identified, including integrins such as $\alpha_7\beta_1$ and $\alpha_5\beta_1$, extracellular matrix components such as fibronectin and laminin, cytosolic proteins (oncogenic RAS), and several T-cell surface receptors, such as CD2/CD3/CD4/CD7/CD43/CD45.²²

Galectin-1 expression has been reported in cultured ECs of different origins, including mouse liver, brain, and lung; rat lung; bovine aorta; and human aorta, umbilical vein, and pulmonary artery.^{9,25-29} Moreover, activation of ECs in vitro results in a rapid increase of galectin-1 expression. For example, treatment of cultured aortic and umbilical vein ECs with low-density lipoprotein (LDL), lipopolysaccharide (LPS), or tumor-conditioned medium

resulted in increased galectin-1 expression at the extracellular side of the cell.^{26,30} Addition of interferon- γ or tumor-conditioned medium induced galectin-1 expression in ECs^{16,31} and increased galectin-1 secretion into the matrix.³⁰ We found that galectin-1 is an early marker of human umbilical vein EC (HUVEC) activation in vitro.⁷ Increased galectin-1 expression also appears to correlate with EC activation in vivo. Galectin-1 is expressed in endothelial venules of activated lymphoid tissue, but not of resting lymph nodes.²⁶ Elevated galectin-1 protein expression was also observed in the ECs of human colon carcinoma and Ewing sarcoma,⁷ in head and neck or lung cancer,²⁵ and in prostate carcinoma.³¹

Galectin-3

Galectin-3 is also an extensively studied galectin family member (reviewed by Dumic et al³²) that is expressed by ECs. The 30-kDa protein consists of a C-terminal CRD and a proline rich N-terminal domain containing several homologous repeats. Binding of the N-terminal domain can result in the formation of oligomers.³³⁻³⁵ The protein can be found both intracellularly and extracellularly, and numerous galectin-3-binding partners have been identified,

Table 1. The galectin family

Name	Prototype*	Tandem repeat	Chimera†	Splice variants‡	Human gene	ENSEMBLE accession number
galectin-1	•	—	—	—	•	ENSG00000100097
galectin-2	•	—	—	—	•	ENSG00000100079
galectin-3	—	—	•	—	•	ENSG00000131981
galectin-4	—	•	—	—	•	ENSG00000171747
galectin-5	•	—	—	—	—	—
galectin-6	—	•	—	—	—	—
galectin-7	•	—	—	—	•	ENSG00000178934
galectin-8	—	•	—	•	•	ENSG00000116977
galectin-9	—	•	—	•	•	ENSG00000168961
galectin-10	•	—	—	—	•	ENSG00000105205
galectin-11	•	—	—	—	—	—
galectin-12	—	•	—	—	•	ENSG00000133317
galectin-13	•	—	—	—	•	ENSG00000105198
galectin-14	•	—	—	•	•	ENSG00000006659
galectin-15	•	—	—	—	—	—

— indicates not applicable.

*Several prototype galectins have been shown to form homodimers (gal-1, -2, and -13) in case of the tandem repeat galectins.

†The only chimera galectin known so far (ie, galectin-3) contains a proline- and glycine-rich C-terminal tail that can mediate oligomerization of the protein.

‡Splicing has been shown to occur in the linker region between the 2 carbohydrate recognition domains (CRDs). For galectin-8 an additional splice variant has been described that results in truncation of the C-terminal CRD.

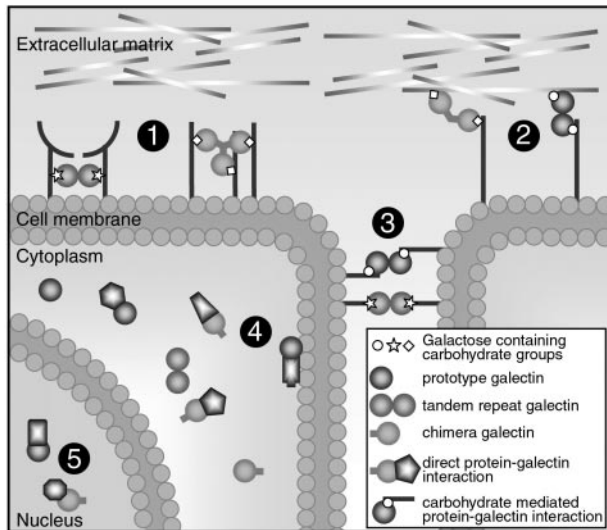


Figure 2. Cellular location and functions of galectins. Galectins can be found in the extracellular space, in the cell membrane, in the cytoplasm, and even in the nucleus. They can bind to proteins via a carbohydrate interaction or via direct protein-protein interactions. Most galectins have multiple functions and they can mediate (1) receptor cross-linking or lattice formation, (2) cell-extracellular matrix interactions, (3) cell-to-cell interactions, (4) intracellular signaling, and (5) posttranscriptional splicing.

including extracellular matrix components such as laminin, fibronectin, vitronectin, and elastin; cell adhesion molecules like N-CAM, lysosome-associated membrane protein (LAMP1)/LAMP2, and integrin $\alpha_3\beta_1$; and cytosolic molecules such as Bcl-2, K-Ras, and annexin VII.³² Galectin-3 can be involved in different biological functions, including cell adhesion and activation, apoptosis, immune regulation, and gene transcription regulation.

Galectin-3 expression has been observed in the cultured ECs of mouse liver, brain, and lung; rat lung; bovine aorta; and human umbilical vein and dermal microvasculature.^{25,29,36} EC expression of galectin-3 *in vivo* has been reported in head and neck and in lung cancer tissues.^{25,37} In a murine tumor model, galectin-3 expression was reported to be approximately 30-fold up-regulated in liver carcinoma-derived ECs compared with normal liver-derived ECs.³⁸

The relationship between the galectin-3 expression level and activation status of ECs is still somewhat unclear. Glinskii et al showed that the interaction between ECs and tumor cells expressing specific carbohydrate structures increases galectin-3 expression in ECs.^{39,40} Recently, it was shown that IL-8 treatment of a hybridoma of HUVECs and epithelioma cells resulted in increased galectin-3 protein levels in the cytoplasm and the cell membrane.⁴¹

Galectin-9

Galectin-9, a more recently discovered galectin family member, is also expressed in ECs. It is a tandem repeat galectin that was first cloned from the tumor tissue of patients with Hodgkin disease.⁴² The protein consists of 2 CRDs that are connected by a linker of about 30 amino acids. Originally, galectin-9 was identified as an eosinophil-specific chemoattractant.⁴³ Recent studies have shown that it can also function as an urate channel.⁴⁴ Known binding partners include type IV collagen⁴⁵ and the Epstein-Barr virus latent membrane protein-1.⁴⁶ Recently, galectin-9 was identified as a receptor for the T helper type 1 (T_H1)-specific cell-surface molecule Tim-3, which is involved in the regulation of the T_H1 response.⁴⁷

In HUVECs, 3 constitutively expressed galectin-9 isoforms have been identified in which either exon 5 or exon 6 are

alternatively spliced.⁴⁸ Stimulation with interferon- γ increases galectin-9 expression in the membrane of HUVECs.⁴⁹ EC expression of galectin-9 has been studied mainly in the context of viral infections. Infection of HUVECs with dengue virus induces galectin-9 mRNA expression.⁵⁰ In addition, treatment of HUVECs with a synthetic mimetic of double-stranded RNA as a model for viral infection increases mRNA and protein expression of galectin-9.⁵¹

Endothelial galectins in tumor progression

As described here, there is clear evidence that ECs express galectin family members and that their expression can be altered during EC activation. The latter observation suggests that galectins contribute to different functions of activated tumor ECs, and that they might be involved in the steps of tumor progression (ie, immune modulation, metastasis, and angiogenesis).

Tumor immune modulation

Infiltration of tissues by immune cells is a multistep process, and ECs play an important role in several of these steps.⁵² The adhesion and rolling of leukocytes along the vessel wall, followed by transendothelial migration of activated leukocytes, are steps in which the endothelium is involved (K. Castermans, A.W.G., Tumor blood vessels, a difficult hurdle for infiltrating leukocytes; manuscript accepted, July 2007). ECs express adhesion molecules that influence leukocyte infiltration and activation (eg, E-selectin, P-selectin, ICAM, and different integrins). While inflammatory cytokines induce the expression of many adhesion molecules, several proangiogenesis factors released by tumor cells counteract the proinflammatory phenotype of ECs by turning off the “immunogenic switch.” This phenomenon is also referred to as EC energy, and enables tumors to escape from immunosurveillance.⁵³⁻⁵⁵ In the last decade, it has become evident that tumor cells can exploit the expression of galectins to manipulate the immune system (for reviews, see Rabinovich et al⁵⁶ and Liu et al¹⁹). Inhibition of galectin-1 expression in murine B16 melanoma cells stimulated a potent tumor-specific T1-type response in mice and a reduction in tumor progression.⁵⁷ Observations in human cancers *in vivo* and human tumor cell lines *in vitro* also suggest a relation between galectin expression and immune evasion.⁵⁸⁻⁶¹ There are also reports that indicate a link between ECs, galectins, and immune regulation. For example, galectin-3 is considered to be a proinflammatory protein since it promotes adhesion of neutrophils to ECs,⁶² acts as a chemoattractant for monocytes and macrophages, and facilitates phagocytosis.³² The interaction of neutrophils with the endothelium stimulates expression of galectin-3 in neutrophils and ECs.⁴¹ Galectin-9 has been shown to be involved in the adhesion of eosinophils to the endothelium.^{43,49} Interferon- γ -induced endothelial expression of galectin-9 is accompanied by enhanced adhesion of human eosinophilic leukemia-1 cells.^{49,51} Recently, binding of galectin-9 to Tim-3 was found to induce the aggregation and death of T_H1 cells *in vitro*. Administration of galectin-9 *in vivo* resulted in suppression of T_H1 autoimmunity, indicating that this interaction could represent a means to inhibit the inflammatory response. It was suggested that interfering with the signaling induced by the interaction might provide new opportunities for pharmacologic interventions.⁶³ However, whether and how galectin-9 expression by ECs might regulate a Tim-3 mediated T_H1 response needs further investigation.

Another role of galectins in the immunoresponse involves regulation of leukocyte survival and function. To infiltrate a tumor tissue, immune cells first have to pass the endothelium, which makes it an obvious site for immunomodulation. Indeed, the presentation of galectin-1 by activated ECs triggers apoptosis of activated T cells.^{16,64,65} Galectin-3 and galectin-9 are also able to induce T-cell apoptosis.⁶⁶⁻⁶⁸ Furthermore, galectin-3 can inhibit T-cell activation by complexing with the T-cell receptor.⁶⁹ Thus, up-regulation of galectin expression by ECs may provide a mechanism to hamper the immune response. Unfortunately, strong evidence showing that these endothelial galectins effectively modulate the tumor immune response *in vivo* is still lacking.

It has also been suggested that EC expression of galectin-1 might mediate an apoptosis independent anti-inflammatory effect on leukocytes. Increased EC expression of galectin-1 was found to decrease transendothelial migration of leukocytes independent of the proapoptotic activity.³⁰ However, in a teratocarcinoma tumor model in galectin-1-null mice, there was no statistically significant change in the number of CD45⁺ or CD8⁺ cells that were found in the tumors of null mice compared with those of wild-type animals.⁷ Since the teratocarcinoma cells express only low levels of galectin-1, the comparable infiltration could not be explained by an effect of the tumor cells. Further research is needed to determine whether and how endothelial galectin-1 influences the *in vivo* immune response.

As described, endothelial expression of galectins might influence processes of the immune response, including leukocyte apoptosis, adhesion, and migration. However, in contrast to our knowledge regarding the immunomodulatory role of galectins expressed by tumor cells, the amount of evidence directly linking endothelial galectins to immune escape is still scarce. The limited data available at present indicate that the possible immunomodulating effects of endothelial galectins depend on multiple aspects, like the expression level and localization, the type and activation status of the interacting cells, and the specific step in the immunogenic cascade. Whether targeting endothelial galectins generates sufficient immunomodulatory effects *in vivo* still needs to be resolved. One of the major challenges for future research will be to distinguish the contribution of endothelial galectins from that of the galectins expressed by the tumor cells. This would be facilitated by the development of animal models that allow conditional and endothelial-specific knockdown of galectin expression. In combination with tumor cells that display different levels of galectin expression, such models could be used to determine whether regulating the endothelial expression of galectins could be used to modulate the antitumor immune response, even in the absence of galectin expression by the tumor cells.

Tumor metastasis formation

The involvement of galectins in both homotypic and heterotypic cell adhesion (reviewed by Elola et al⁷⁰) has been suggested to facilitate tumor metastasis. Galectin expression by tumor cells can play a role in different steps of metastasis such as cell detachment from the primary tumor site, diapedesis into vessels, and cell adhesion to ECs at distant sites.^{19,70,71} For example, high expression of galectin-3 by metastatic MDA-MB-435 breast carcinoma cells increased their adhesion to monolayers of ECs.⁷² At these heterotypic adhesion sites, homotypic cell aggregation was also observed, not only for breast carcinoma cells but also for DU-145 prostate carcinoma cells,⁴⁰ which also express high levels of galectin-3. Moreover, clustering of galectin-3 occurred at the cell-contact

sites^{40,73} suggesting that expression of this galectin can play a role in metastasis formation (for review, see Takenaka et al⁷¹). However, metastasis formation is a complex process, and the expression of galectins by tumor cells can have different effects that likely depend on factors like the tumor cell type, the specific step in the metastatic process, and the level of expression. This complicates the study of their function and could account for the sometimes-conflicting data when correlating galectin expression in tumors and tumor progression.

As expected from their multifunctional properties, the contribution of galectins to metastasis is not restricted to the adhering cell (tumor cell) but also involves the cell that is adhered to (EC). Lotan et al observed that the percentage of metastatic large-cell lymphoma cells that attached to either cultured liver or lung ECs reflected the organ preference for metastasis.²⁵ Similar observations were reported when the adhesion of several different tumor cell lines to human bone marrow ECs and HUVECs was studied.⁷⁴ Tumor cells preferentially adhered to bone marrow ECs. Moreover, the adhesion of PC3 prostate carcinoma cells was inhibited after preincubation of the ECs with anti-galectin-3 antibody.⁷⁴ In a study by Clause et al, culturing ECs in PC-3-conditioned medium induced galectin-1 expression, which resulted in increased attachment of PC-3 cells. This increased attachment was inhibited by pretreating the ECs with an anti-galectin-1 antibody.³¹ Apparently, the preference of tumor cells to bind to specific ECs involves the expression of galectins by the endothelium. The observation that the expression of endothelial galectins can be influenced by heterotypic cell-cell contact or by molecules secreted by tumor cells suggests an involvement of endothelial galectins in organ-specific tumor metastasis.

A possible mechanism by which endothelial galectins appear to contribute to metastasis involves binding to carbohydrate ligands presented by tumor cells. It was suggested 20 years ago that increased levels of beta1,6-branched *N*-linked oligosaccharides increased the metastatic potential of tumor cells.⁷⁵ Thus, altered glycosylation and exposure of glycoproteins by tumor cells might facilitate their adhesion to endothelial galectins. For example, Kuźbicki et al as well as others observed increased expression and membrane translocation of LAMP1 in metastatic melanoma cells.^{37,76,77} LAMP1 contains high levels of carbohydrate moieties that bind galectin-3.^{77,78} Thus, elevated expression of galectin-3 by activated ECs could facilitate the binding of those tumor cells that expose LAMP1. In line with this, it was observed that preincubation of frozen lung tumor sections with lactose partially inhibited the adhesion of melanoma cells.³⁷ Sarafian et al used lactose to inhibit galectin-3 binding to metastasizing LAMP1-expressing melanoma cells.⁷⁷ Although the inhibitory effects of lactose could be caused by the inhibition of other galectin-binding glycoproteins, the observation that the metastatic tumor cells displayed increased translocation of LAMP1 strongly suggest a causal relationship.

Another galectin-binding carbohydrate that has been described to facilitate adhesion of cancer cells to the endothelium is the Thomsen-Friedenreich glycoantigen (TFag; recently reviewed by Yu⁷⁹). High expression of TFag is associated with most human cancers, and at least 2 surface molecules are known to present TFag (ie, CD44v6 and the transmembrane mucin MUC1). It was shown that interactions between ECs and TFag-expressing tumor cells induces endothelial expression of galectin-3.^{39,40,72} In addition, it was found that endothelial adhesion of tumor cells that display TFag on MUC1 is facilitated by circulating galectin-3.⁸⁰ Furthermore, blocking either TFag or galectin-3 could prevent the *in vivo* formation of breast and prostate carcinoma metastasis.^{10,73,81} All

these data suggest that specific glycosylations on tumor cells provide ligands for galectin-mediated metastasis.

However, galectins are not the only molecules that can mediate adhesion between tumor cells and ECs. Saitoh et al compared the composition of Asn-linked oligosaccharides on LAMP1 and LAMP2 after immunoprecipitation from different colon carcinoma cells. They observed an increased expression of both proteins in highly metastatic cells that correlated with an increase in sialyl Lewis X carbohydrates.⁸² Further research indicated that sialyl Lewis X mediated the adhesion of the metastatic cells to activated ECs via E-selectin.⁸³ Integrins have also been associated with heterotypic cell adhesion and tumor metastasis.⁸⁴ Conclusively, the tumor ECs express a panel of molecules, including galectins, that can facilitate tumor metastasis. Unraveling the interplay between all these molecules and their ligands provides a challenge for future research. Furthermore, targeting the metastatic molecules that reside on the activated endothelium (eg, galectins) opens the possibility to develop treatment strategies that prevent or delay metastasis formation in patients with cancer, independent of the tumor type.

Tumor angiogenesis

Upon activation by growth factors produced by the tumor, ECs in nearby vessels embark on a cascade of events that results in the formation and growth of a mature tumor vasculature. This involves remodeling of the extracellular matrix, proliferation and migration of the ECs, formation of tubelike structures, and, eventually, maturation of the newly formed vessels.² Given their multifunctionality, galectins are likely to play a role in several of these steps. Exogenously added galectin-3 was shown to induce *in vitro* tube formation in a carbohydrate-dependent manner.⁸⁵ Tube formation and EC migration is also stimulated by the extracellular domain of NG2 proteoglycan, of which the proangiogenesis potential is thought to be mediated by galectin-3.³⁶ We observed that knock-down of galectin-1 expression in cultured ECs inhibited cell proliferation and migration.⁷

A strong genetic argument linking galectins to tumor angiogenesis was recently obtained from experiments *in vivo*. When tumor cells were injected in wild-type and galectin-1-null mice, tumor growth was severely hampered in the absence of galectin-1. This growth delay was accompanied by a 4-fold decrease in microvessel density.⁷ The observation that the angiogenesis-independent onset of tumor growth was similar in wild-type and null mice, together with an unaltered tumor immune response, strongly suggested that the observed effects were mainly caused by decreased tumor angiogenesis.⁷ Future research using different galectin-null mice in combination with different tumor models will gain further insights in the exact role of galectins in tumor angiogenesis.

Knocking-down galectin-1 expression during zebrafish development resulted in abnormal and irregular vessel growth, suggesting that EC migration and vessel guidance are dependent on galectin-1.⁷ In line with this, Nangia-Makker and colleagues showed that both *in vitro* and *in vivo*, galectin-3 acts as a chemoattractant for ECs.^{85,86} Galectin-9 has also been reported as a chemoattractant, although the direct effects of galectin-9 on EC migration have not been studied. Nevertheless, current observations suggest that galectins might be involved in migration and correct pathfinding of ECs. In the last few years, there have been many reports on proteins that share a regulatory activity in vascular and neuronal network formation (eg, *robos*/slits, *ephs*/*ephrins*, *neuropilins*/*semaphorins*,

and *unc5B*/*netrins*).^{87,88} There are also several reports that galectin-1 plays an important role in axonal targeting. Galectin-1 was shown to stimulate the *in vitro* outgrowth of dorsal root ganglion (DRG) neurons and olfactory axons, to promote Schwann cell migration, and to enhance the regeneration of sensory and motor neurons *in vivo*.⁸⁹⁻⁹¹ The galectin-1-null mice display defects that are consistent with a role for this lectin in neuronal pathfinding.^{89,90,92} For example, a subset of primary sensory olfactory axons fail to reach their appropriate target site in the absence of galectin-1.⁹² Based on these observations, together with the suggested role of galectin-1 in vascular guidance, it is tempting to speculate that galectin-1 is also a member of the group of proteins that regulate patterning of both vascular and neuronal networks. However, it remains to be elucidated how the apparent normal vascular development is regulated in mice that lack galectin-1 or even both galectin-1 and galectin-3. It might be that, similar to the neuronal growth and regeneration, small vascular abnormalities do occur in these mice that only become apparent after a pathophysiologic challenge such as cancer.

Altogether, the current literature indicates that galectins, and most notably galectin-1, are involved in different endothelial processes during angiogenesis, and that they can regulate tumor angiogenesis. Although the exact function and involvement of other galectins in angiogenesis still remains to be studied, it is likely that interfering with galectin function might be used to obstruct tumor angiogenesis.

Exploiting galectins in the tumor endothelium for combined cancer therapy

As described here, members of the galectin family are expressed by ECs and possibly contribute to tumor progression (Figure 3). This makes galectins in the tumor endothelium promising targets for cancer therapy. Lotan and coworkers showed that preincubation of liver ECs with an anti-galectin-1 antibody partially inhibited the attachment of liver-metastatic large-cell lymphoma cells *in vitro*.²⁵ A similar observation was made by Clause et al, who were able to inhibit the attachment of prostate carcinoma cells to cultured ECs by preincubating the latter with an anti-galectin-1 antibody.³¹ Thus, blocking galectin-1 might prevent heterotypic adhesion between tumor cells and ECs, thereby hampering metastasis. Unfortunately, the only *in vivo* study with a tumor model in galectin-1-null mice (ie, F9 teratocarcinoma cells) did not address tumor metastasis. The colonization of either lung or liver by the teratocarcinoma cells used in that study has been shown to depend on the cell differentiation status,⁹³ and future experiments could disclose the role of endothelial galectin-1 in this process. Furthermore, experiments using different *in vivo* metastasis models could disclose the potential therapeutic applications of galectin-1-blocking agents on tumor metastasis.

For galectin-3, more evidence exists that it might be a target for antimetastatic cancer treatment. Peptide antagonists that specifically bind the CRD of galectin-3 were recently shown to inhibit the adhesion of MDA-MB-435 breast carcinoma cells to cultured EC monolayers.¹⁰ Blocking galectin-3 with lactose significantly inhibits the LAMP1-mediated adhesion of B16 melanoma cells to galectin-3 expressing lung ECs.³⁷ These *in vitro* observations suggest that blocking specific galectins might hamper metastasis formation *in vivo*. Indeed, Glinskii et al demonstrated that blocking galectin-3 with antibodies inhibited the accumulation of metastatic cells in the lungs and bones of mice.⁸¹ Antimetastatic activity was

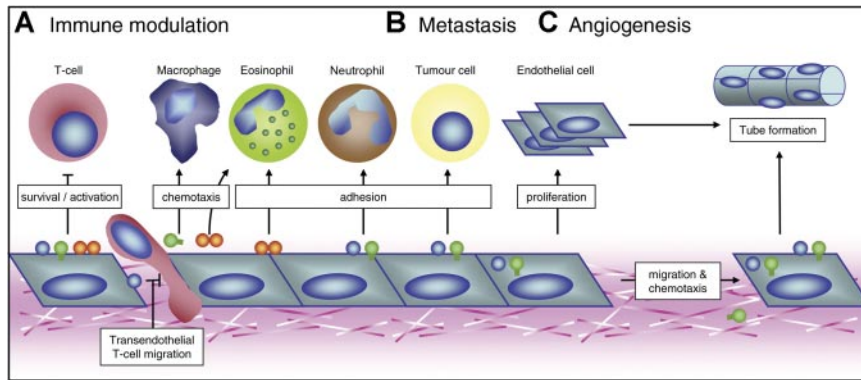


Figure 3. Possible roles of endothelial galectins in 3 mainstays of tumorigenesis. Schematic overview of the mechanisms by which galectin expression by activated ECs might contribute to 3 key processes in tumor progression. (A) Galectin-1, galectin-3, and galectin-9 can modulate the antitumor immune response both positively and negatively. They mainly regulate T-cell survival, activation, and transendothelial migration. In addition, they could facilitate adhesion of neutrophils and eosinophils or act as chemoattractants for macrophages and eosinophils. The overall effect possibly allows tumor cells to escape from immune surveillance. (B) Endothelial galectins have also been suggested to facilitate metastasis formation by promoting heterotypic cell adhesion between circulating tumor cells and ECs. Furthermore, the adhesion of circulating cells to ECs could stimulate the endothelial expression of galectin-1 and galectin-3. (C) Galectin-1 and galectin-3 have been shown to be involved in tumor angiogenesis. They can influence EC migration and proliferation, thereby promoting tube formation. In addition, galectin-3 acts as a chemoattractant for ECs.

also reported for another molecule that binds galectin-3, a modified form of citrus pectin. Modified citrus pectin (MCP), a galactosyl-rich polysaccharide, inhibits the *in vitro* adhesion of rat prostate cancer MAT-LyLu cells to rat ECs.⁹⁴ While MCP had no effect on the growth of the primary tumor, rats with at least 0.1% MCP (wt/vol) in their drinking water displayed a significant decrease in the number of lung metastatic colonies.⁹⁴ Comparable observations were reported using metastatic human breast carcinoma cells. Following injection of human metastatic tumor cells in mice on a MCP-rich diet, a decrease in lung metastasis was observed.⁸⁶ Similar to galectin-1, studies in galectin-3-null mice might be used to further address the role of endothelial galectin-3 in tumor metastasis. In the latter study, there was also a significant decrease in tumor microvessel density, indicating that blocking galectin-3 had a direct effect on tumor angiogenesis.⁸⁶ Rabinovich et al have shown that blocking galectins can indeed be an effective strategy to inhibit tumor angiogenesis.²⁹ They developed synthetic lactulose amines that inhibited the binding of galectin-1 and galectin-3 to glycosylated 90K protein. These compounds selectively modulated tumor cell aggregation and apoptosis, and were able to inhibit *in vitro* tube formation of ECs. Others have tried anti-galectin antibodies to interfere with galectin function in angiogenesis. However, the effects of these antibodies in *in vitro* angiogenesis assays are diverse, ranging from inhibition to no response, or even reversal of the inhibitory effects of synthetic blockers.^{29,36,85} These differences are most likely related to variations in the antibodies used, the experimental conditions, and the reading output.

We identified galectin-1 as an important target to inhibit tumor angiogenesis when screening for anginex receptors.⁷ Anginex, which is a synthetic 33-mer peptide that specifically homes to the tumor vasculature, inhibits tumor angiogenesis by inhibition of EC proliferation and migration. Secondary to this, EC death occurs through anoikis.⁹⁵⁻⁹⁷ It was suggested that anginex uses soluble fibronectin to home to the tumor vasculature.⁹⁷ Using the artificial anginex-encoding gene⁹⁸ in a yeast 2-hybrid analysis, we identified galectin-1 as the main binding partner in activated ECs. Indeed, interfering with endothelial expression of galectin-1 or treatment with anti-galectin-1 antibodies *in vitro* induced similar angiostatic effects compared with treatment with anginex.⁷ Furthermore, tumors in galectin-1-null mice were no longer responsive to anginex therapy, confirming that the antiangiogenesis activity of the peptide was mediated through galectin-1.⁷ This is in contradic-

tion with the finding that anginex directly disrupts the EC membrane.⁹⁹ However, the latter study was performed on cultured ECs *in vitro*, outside the context of a tumor vasculature.

Whether and how targeting of galectins in the endothelium affects the antitumor immune response is less well studied. Given the observation that increased expression of galectins could have immunosuppressive effects, interfering with galectin function might counteract the galectin-mediated tumor immune escape. In line with this, Sato et al showed that lactose treatment inhibited the galectin-3-mediated adhesion of neutrophils to ECs *in vitro*.⁶² In addition, we observed that incubation of cultured ECs with a galectin-1-blocking antibody restored the transendothelial migration of T cells.³⁰ Thus, apart from preventing leukocyte apoptosis, blocking galectins in the endothelium might lead to an enhanced leukocyte infiltration of the tumor. However, in a tumor model in galectin-1-null mice, the infiltration of leukocytes was not different from tumors in wild-type animals.⁷ As described, studies using *in vivo* tumor models that can distinguish between the effects of endothelial galectins and galectins expressed by the tumor cells represent one of the challenges for future research.

Concluding remarks and future perspectives

In this review, we have summarized the current knowledge regarding the expression of galectins in ECs and their possible roles in cancer. It is well established that ECs are involved in several steps of tumor progression and that they provide a target for tumor-type independent treatment strategies. ECs express at least 3 members of the galectin family, and their expression is regulated during cell activation. Whether other galectins are also expressed by ECs, and how their expression relates to EC activation, remains to be investigated. The observation that endothelial galectin expression differs upon cell activation might be used to target activated ECs for diagnostic purposes in a similar fashion as $\alpha_v\beta_3$ or E-selectin targeting.¹⁰⁰ The elevated expression of galectins on the surface of activated ECs could also open a window of opportunity to develop strategies for targeted delivery of liposomes carrying drugs or viral vectors for gene therapy.⁴ In line with this, we recently provided evidence that liposomes conjugated with the galectin-1-binding peptide anginex can indeed be used to target fluorescently labeled paramagnetic liposomes to activated ECs

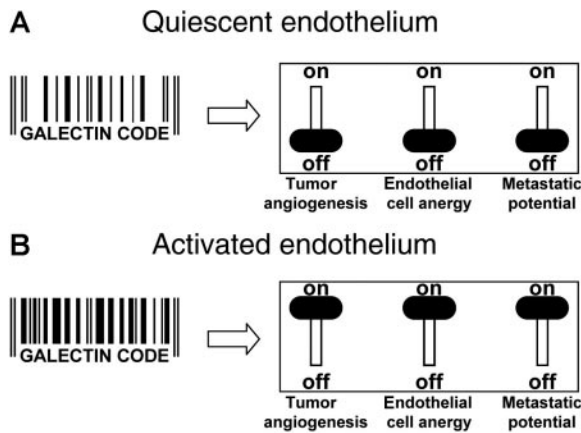


Figure 4. Targeting galectins in the tumor endothelium. (A) In quiescent ECs, the specific set of galectins, their expression level, and their cellular localization is represented by a “galectin code.” Having this galectin code, the ECs are angiogenically inactive, the immune surveillance is not impaired, and heterotypic cell adhesion of circulating cells is hampered. The net result is an environment that does not promote tumor progression. (B) In the presence of stimulatory growth factors, the ECs become activated, causing alterations in the repertoire, expression level, and even localization of galectins. This change in the galectin code enhances the angiogenic potential of the cells, hampers a proper immune response, and facilitates metastasis formation. Consequently, the tumor progression potential is high. From this, it can be speculated that changing the galectin code (eg, by altering galectin expression levels or by directly targeting the galectins with blocking antibodies or peptides), a combination of effects could be generated. This might involve inhibition of angiogenesis, reversal of EC energy, and/or prevention of metastasis formation. Thus, the progressive phenotype of any tumor might be reversed by targeting the galectins that are present on the ECs.

in vitro.¹⁰¹ Future experiments using different in vivo tumor models will have to determine the feasibility to apply such an approach for diagnostic imaging of tumor angiogenesis in human patients with cancer.

Beyond the possible applications in tumor vascular targeting, galectins in the endothelium are also suggested to play a role in key steps of cancer progression, including tumor angiogenesis, tumor immune escape, and tumor metastasis. As described, the galectin type, expression level, and even cellular localization can be altered upon EC activation. It can be hypothesized that alterations in the

“galectin code” of ECs provides an excellent target for EC-based therapy, especially since such therapies might be tumor-type independent and less susceptible to drug-induced resistance (Figure 4). In fact, targeting endothelial galectins has already been shown to induce a therapeutic effect on tumor immunity, tumor metastasis, and tumor angiogenesis. Several compounds have been described that interfere with or completely block galectin function, including antibodies, peptides, and chemical-binding substrates. Recently, an approach was presented that allows the identification of glycopeptides that bind to and possibly inhibit galectins.¹⁰² Different libraries of fully randomized series of peptide hexamers were generated using both unsubstituted and glycosylated amino acids. Following screening against fluorescently labeled galectin-1 and galectin-3, the interacting hexa(glyco)peptides were further characterized, and their ability of inhibit galectin-binding to tumor cells was determined. Some of the interacting hexa(glyco)peptides were more effective inhibitors than lactose, suggesting that the inhibitory activity also involved binding of the nonsugar part of the glycopeptide.¹⁰² A better understanding of the inhibitory mechanisms of galectin-binding compounds might lead to the development of more specific and potent inhibitors. It is tempting to speculate that blocking a single galectin with 1 of those compounds could provoke a response in different steps of tumor progression, thereby preventing tumor metastasis, inhibiting tumor angiogenesis, and reversing tumor immune escape. However, for the rational development of galectin-based therapeutic and diagnostic applications, more insights in their exact function in endothelial biology is of utmost importance.

Authorship

Contribution: V.L.J.L.T., F.P., L.G.B., and A.W.G. wrote the paper.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Arjan W. Griffioen, Department of Pathology, Angiogenesis Laboratory, Maastricht University & University Hospital Maastricht, PO Box 5800, 6202 AZ Maastricht, the Netherlands; e-mail: aw.griffioen@path.unimaas.nl.

References

- Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med*. 1971;285:1182-1186.
- Griffioen AW, Molema G. Angiogenesis: potentials for pharmacologic intervention in the treatment of cancer, cardiovascular diseases, and chronic inflammation. *Pharmacol Rev*. 2000;52:237-268.
- Burrows FJ, Thorpe PE. Eradication of large solid tumors in mice with an immunotoxin directed against tumor vasculature. *Proc Natl Acad Sci U S A*. 1993;90:8996-9000.
- Brandwijk RJ, Griffioen AW, Thijssen VL. Targeted gene-delivery strategies for angiostatic cancer treatment. *Trends Mol Med*. 2007;13:200-209.
- Neri D, Bicknell R. Tumour vascular targeting. *Nat Rev Cancer*. 2005;5:436-446.
- van Beijnum JR, Griffioen AW. In silico analysis of angiogenesis associated gene expression identifies angiogenic stage related profiles. *Biochim Biophys Acta*. 2005;1755:121-134.
- Thijssen VL, Postel R, Brandwijk RJ, et al. Galectin-1 is essential in tumor angiogenesis and is a target for antiangiogenesis therapy. *Proc Natl Acad Sci U S A*. 2006;103:15975-15980.
- Bidon-Wagner N, Le Pennec JP. Human galectin-8 isoforms and cancer. *Glycoconj J*. 2004;19:557-563.
- Rabinovich GA. Galectin-1 as a potential cancer target. *Br J Cancer*. 2005;92:1188-1192.
- Zou J, Glinesky VV, Landon LA, Matthews L, Deutscher SL. Peptides specific to the galectin-3 carbohydrate recognition domain inhibit metastasis-associated cancer cell adhesion. *Carcinogenesis*. 2005;26:309-318.
- Cooper DN. Galectinomics: finding themes in complexity. *Biochim Biophys Acta*. 2002;1572:209-231.
- Seelenmeyer C, Wegehling S, Tews I, Kunzler M, Aebi M, Nickel W. Cell surface counter receptors are essential components of the unconventional export machinery of galectin-1. *J Cell Biol*. 2005;171:373-381.
- Hughes RC. Galectins as modulators of cell adhesion. *Biochimie*. 2001;83:667-676.
- Lefler H. Galectins structure and function: a synopsis. *Results Probl Cell Differ*. 2001;33:57-83.
- Houzelstein D, Goncalves IR, Fadden AJ, et al. Phylogenetic analysis of the vertebrate galectin family. *Mol Biol Evol*. 2004;21:1177-1187.
- Perillo NL, Pace KE, Seilhamer JJ, Baum LG. Apoptosis of T cells mediated by galectin-1. *Nature*. 1995;378:736-739.
- Park JW, Voss PG, Grabski S, Wang JL, Patterson RJ. Association of galectin-1 and galectin-3 with Gemin4 in complexes containing the SMN protein. *Nucleic Acids Res*. 2001;29:3595-3602.
- Scott K, Weinberg C. Galectin-1: a bifunctional regulator of cellular proliferation. *Glycoconj J*. 2004;19:467-477.
- Liu FT, Rabinovich GA. Galectins as modulators of tumour progression. *Nat Rev Cancer*. 2005;5:29-41.
- van den Brule F, Califice S, Castronovo V. Expression of galectins in cancer: a critical review. *Glycoconj J*. 2004;19:537-542.
- Huflejt ME, Lefler H. Galectin-4 in normal tissues and cancer. *Glycoconj J*. 2004;20:247-255.
- Camby I, Le Mercier M, Lefranc F, Kiss R. Galectin-1: a small protein with major functions. *Glycobiology*. 2006;16:137R-157R.
- Elola MT, Chiesa ME, Alberti AF, Mordoh J, Fink NE. Galectin-1 receptors in different cell types. *J Biomed Sci*. 2005;12:13-29.
- Perillo NL, Marcus ME, Baum LG. Galectins: versatile modulators of cell adhesion, cell proliferation, and cell death. *J Mol Med*. 1998;76:402-412.
- Lotan R, Belloni PN, Tressler RJ, Lotan D, Xu XC, Nicolson GL. Expression of galectins on microvessel endothelial cells and their involvement

- in tumour cell adhesion. *Glycoconj J*. 1994;11:462-468.
26. Baum LG, Seilhamer JJ, Pang M, Levine WB, Beynon D, Berliner JA. Synthesis of an endogenous lectin, galectin-1, by human endothelial cells is up-regulated by endothelial cell activation. *Glycoconj J*. 1995;12:63-68.
27. Lame MW, Jones AD, Wilson DW, Dunston SK, Segall HJ. Protein targets of monocrotaline pyrrole in pulmonary artery endothelial cells. *J Biol Chem*. 2000;275:29091-29099.
28. Lame MW, Jones AD, Wilson DW, Segall HJ. Monocrotaline pyrrole targets proteins with and without cysteine residues in the cytosol and membranes of human pulmonary artery endothelial cells. *Proteomics*. 2005;5:4398-4413.
29. Rabinovich GA, Cumashi A, Bianco GA, et al. Synthetic lactulose amines: novel class of anti-cancer agents that induce tumor-cell apoptosis and inhibit galectin-mediated homotypic cell aggregation and endothelial cell morphogenesis. *Glycobiology*. 2006;16:210-220.
30. He J, Baum LG. Endothelial cell expression of galectin-1 induced by prostate cancer cells inhibits T-cell transendothelial migration. *Lab Invest*. 2006;86:578-590.
31. Clausse N, van den Brule F, Waltregny D, Garnier F, Castronovo V. Galectin-1 expression in prostate tumor-associated capillary endothelial cells is increased by prostate carcinoma cells and modulates heterotypic cell-cell adhesion. *Angiogenesis*. 1999;3:317-325.
32. Dumic J, Dabelic S, Flogel M. Galectin-3: An open-ended story. *Biochim Biophys Acta*. 2006;1760:616-635.
33. Massa SM, Cooper DN, Leffler H, Barondes SH. L-29, an endogenous lectin, binds to glycoconjugate ligands with positive cooperativity. *Biochemistry*. 1993;32:260-267.
34. Ahmad N, Gabius HJ, Andre S, et al. Galectin-3 precipitates as a pentamer with synthetic multivalent carbohydrates and forms heterogeneous cross-linked complexes. *J Biol Chem*. 2004;279:10841-10847.
35. Nieminen J, Kuno A, Hirabayashi J, Sato S. Visualization of galectin-3 oligomerization on the surface of neutrophils and endothelial cells using fluorescence resonance energy transfer. *J Biol Chem*. 2006;282:1374-1383.
36. Fukushi J, Makagiarsan IT, Stallcup WB. NG2 proteoglycan promotes endothelial cell motility and angiogenesis via engagement of galectin-3 and alpha3beta1 integrin. *Mol Biol Cell*. 2004;15:3580-3590.
37. Krishnan V, Bane SM, Kawle PD, Naresh KN, Kalraia RD. Altered melanoma cell surface glycosylation mediates organ specific adhesion and metastasis via lectin receptors on the lung vascular endothelium. *Clin Exp Metastasis*. 2005;22:11-24.
38. Ryschich E, Lizdenis P, Ittrich C, et al. Molecular fingerprinting and autocrine growth regulation of endothelial cells in a murine model of hepatocellular carcinoma. *Cancer Res*. 2006;66:198-211.
39. Glinkskii OV, Turk JR, Pienta KJ, Huxley VH, Glinksky VV. Evidence of porcine and human endothelium activation by cancer-associated carbohydrates expressed on glycoproteins and tumour cells. *J Physiol*. 2004;554:89-99.
40. Glinksky VV, Glinksky GV, Glinkskii OV, et al. Intravascular metastatic cancer cell homotypic aggregation at the sites of primary attachment to the endothelium. *Cancer Res*. 2003;63:3805-3811.
41. Gil CD, La M, Perretti M, Oliani SM. Interaction of human neutrophils with endothelial cells regulates the expression of endogenous proteins annexin 1, galectin-1 and galectin-3. *Cell Biol Int*. 2006;30:338-344.
42. Tureci O, Schmitt H, Fadle N, Pfreundschuh M, Sahin U. Molecular definition of a novel human galectin which is immunogenic in patients with Hodgkin's disease. *J Biol Chem*. 1997;272:6416-6422.
43. Matsumoto R, Matsumoto H, Seki M, et al. Human ealectin, a variant of human galectin-9, is a novel eosinophil chemoattractant produced by T lymphocytes. *J Biol Chem*. 1998;273:16976-16984.
44. Lipkowitz MS, Leal-Pinto E, Cohen BE, Abramson RG. Galectin 9 is the sugar-regulated urate transporter/channel UAT. *Glycoconj J*. 2004;19:491-498.
45. Irie A, Yamauchi A, Kontani K, et al. Galectin-9 as a prognostic factor with antimetastatic potential in breast cancer. *Clin Cancer Res*. 2005;11:2962-2968.
46. Pioche-Durieu C, Keryer C, Souquere S, et al. In nasopharyngeal carcinoma cells, Epstein-Barr virus LMP1 interacts with galectin 9 in membrane raft elements resistant to simvastatin. *J Virol*. 79:13326-13337, 2005.
47. Zhu C, Anderson AC, Schubart A, et al. The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity. *Nat Immunol*. 2005;6:1245-1252.
48. Spitzenberger F, Graessler J, Schroeder HE. Molecular and functional characterization of galectin 9 mRNA isoforms in porcine and human cells and tissues. *Biochimie*. 2001;83:851-862.
49. Imaizumi T, Kumagai M, Sasaki N, et al. Interferon- γ stimulates the expression of galectin-9 in cultured human endothelial cells. *J Leukoc Biol*. 2002;72:486-491.
50. Warke RV, Xhaja K, Martin KJ, et al. Dengue virus induces novel changes in gene expression of human umbilical vein endothelial cells. *J Virol*. 2003;77:11822-11832.
51. Ishikawa A, Imaizumi T, Yoshida H, et al. Double-stranded RNA enhances the expression of galectin-9 in vascular endothelial cells. *Immunol Cell Biol*. 2004;82:410-414.
52. Almkvist J, Karlsson A. Galectins as inflammatory mediators. *Glycoconj J*. 2004;19:575-581.
53. Griffioen AW, Damen CA, Blijham GH, Groenewegen G. Tumor angiogenesis is accompanied by a decreased inflammatory response of tumor-associated endothelium. *Blood*. 1996;88:667-673.
54. Molema G, Griffioen AW. Rocking the foundations of solid tumor growth by attacking the tumor's blood supply. *Immunol Today*. 1998;19:392-394.
55. Dirks AE, Oude Egbrink MG, Kuijpers MJ, et al. Tumor angiogenesis modulates leukocyte-vessel wall interactions in vivo by reducing endothelial adhesion molecule expression. *Cancer Res*. 2003;63:2322-2329.
56. Rabinovich GA, Toscano MA, Ilarregui JM, Rubinstein N. Shedding light on the immunomodulatory properties of galectins: novel regulators of innate and adaptive immune responses. *Glycoconj J*. 2004;19:565-573.
57. Rubinstein N, Alvarez M, Zwirner NW, et al. 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*. 2004;5:241-251.
58. Le QT, Shi G, Cao H, et al. Galectin-1: a link between tumor hypoxia and tumor immune privilege. *J Clin Oncol*. 2005;23:8932-8941.
59. Gandhi MK, Moll G, Smith C, et al. Galectin-1 mediated suppression of EBV-specific T-cell immunity in classical Hodgkin's Lymphoma. *Blood*. Prepublished online April 16, 2007, as DOI 10.1182/blood-2007-01-066100.
60. Valenzuela HF, Pace KE, Cabrera PV, et al. O-glycosylation regulates LNCaP prostate cancer cell susceptibility to apoptosis induced by galectin-1. *Cancer Res*. 2007;67:6155-6162.
61. Zubieta MR, Furman D, Barrio M, Bravo AI, Domenichini E, Mordoh J. Galectin-3 expression correlates with apoptosis of tumor-associated lymphocytes in human melanoma biopsies. *Am J Pathol*. 2006;168:1666-1675.
62. Sato S, Ouellet N, Pelletier I, Simard M, Rancourt A, Bergeron MG. Role of galectin-3 as an adhesion molecule for neutrophil extravasation during streptococcal pneumonia. *J Immunol*. 2002;168:1813-1822.
63. van de Weyer PS, Muehlefeit M, Klose C, Bonventre JV, Walz G, Kuehn EW. A highly conserved tyrosine of Tim-3 is phosphorylated upon stimulation by its ligand galectin-9. *Biochem Biophys Res Commun*. 2006;351:571-576.
64. Perillo NL, Uittenbogaart CH, Nguyen JT, Baum LG. Galectin-1, an endogenous lectin produced by thymic epithelial cells, induces apoptosis of human thymocytes. *J Exp Med*. 1997;185:1851-1858.
65. He J, Baum LG. Presentation of galectin-1 by extracellular matrix triggers T cell death. *J Biol Chem*. 2004;279:4705-4712.
66. Wada J, Ota K, Kumar A, Wallner EI, Kanwar YS. Developmental regulation, expression, and apoptotic potential of galectin-9, a beta-galactoside binding lectin. *J Clin Invest*. 1997;99:2452-2461.
67. Fukumori T, Takenaka Y, Yoshii T, et al. CD29 and CD7 mediate galectin-3-induced type II T-cell apoptosis. *Cancer Res*. 2003;63:8302-8311.
68. Dai SY, Nakagawa R, Itoh A, et al. Galectin-9 induces maturation of human monocyte-derived dendritic cells. *J Immunol*. 2005;175:2974-2981.
69. Demetriou M, Granovsky M, Quaggin S, Dennis JW. Negative regulation of T-cell activation and autoimmunity by Mgal5 N-glycosylation. *Nature*. 2001;409:733-739.
70. Elola MT, Wolfenstein-Todel C, Troncoso P, Vasta GR, Rabinovich GA. Galectins: matricellular glycan-binding proteins linking cell adhesion, migration, and survival. *Cell Mol Life Sci*. 2007;64:1679-1700.
71. Takenaka Y, Fukumori T, Raz A. Galectin-3 and metastasis. *Glycoconj J*. 2004;19:543-549.
72. Khaldooyanidi SK, Glinksky VV, Sikora L, et al. 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*. 2003;278:4127-4134.
73. Glinksky VV, Huflejt ME, Glinksky GV, Deutscher SL, Quinn TP. Effects of Thomsen-Friedenreich antigen-specific peptide P-30 on beta-galactoside-mediated homotypic aggregation and adhesion to the endothelium of MDA-MB-435 human breast carcinoma cells. *Cancer Res*. 2000;60:2584-2588.
74. Lehr JE, Pienta KJ. Preferential adhesion of prostate cancer cells to a human bone marrow endothelial cell line. *J Natl Cancer Inst*. 1998;90:118-123.
75. Dennis JW, Laferte S, Waghorne C, Breitman ML, Kerbel RS. Beta 1-6 branching of Asn-linked oligosaccharides is directly associated with metastasis. *Science*. 1987;236:582-585.
76. Kuzbicki L, Gajo B, Chirot BW. Different expression of lysosome-associated membrane protein-1 in human melanomas and benign melanocytic lesions. *Melanoma Res*. 2006;16:235-243.
77. Sarafian V, Jadot M, Foidart JM, et al. Expression of Lamp-1 and Lamp-2 and their interactions with galectin-3 in human tumor cells. *Int J Cancer*. 1998;75:105-111.
78. Inohara H, Raz A. Identification of human melanoma cellular and secreted ligands for galectin-3. *Biochem Biophys Res Commun*. 1994;201:1366-1375.
79. Yu LG. The oncofetal Thomsen-Friedenreich carbohydrate antigen in cancer progression. *Glycoconj J*. Prepublished on April 25, 2007, as DOI 10.1007/s10719-007-9034-3.

80. Yu LG, Andrews N, Zhao Q, et al. Galectin-3 interaction with Thomsen-Friedenreich disaccharide on cancer-associated MUC1 causes increased cancer cell-endothelial adhesion. *J Biol Chem.* 2007;282:773-781.
81. Glinskii OV, Huxley VH, Glinsky GV, Pienta KJ, Raz A, Glinsky VV. Mechanical entrapment is insufficient and intercellular adhesion is essential for metastatic cell arrest in distant organs. *Neoplasia.* 2005;7:522-527.
82. Saitoh O, Wang WC, Lotan R, Fukuda M. Differential glycosylation and cell surface expression of lysosomal membrane glycoproteins in sublines of a human colon cancer exhibiting distinct metastatic potentials. *J Biol Chem.* 1992;267:5700-5711.
83. Sawada R, Tsuboi S, Fukuda M. Differential E-selectin-dependent adhesion efficiency in sublines of a human colon cancer exhibiting distinct metastatic potentials. *J Biol Chem.* 1994;269:1425-1431.
84. Hood JD, Cheresh DA. Role of integrins in cell invasion and migration. *Nat Rev Cancer.* 2002;2:91-100.
85. Nangia-Makker P, Honjo Y, Sarvis R, et al. Galectin-3 Induces Endothelial Cell Morphogenesis and Angiogenesis. *Am J Pathol.* 2000;156:899-909.
86. Nangia-Makker P, Hogan V, Honjo Y, et al. Inhibition of human cancer cell growth and metastasis in nude mice by oral intake of modified citrus pectin. *J Natl Cancer Inst.* 2002;94:1854-1862.
87. Klagsbrun M, Eichmann A. A role for axon guidance receptors and ligands in blood vessel development and tumor angiogenesis. *Cytokine Growth Factor Rev.* 2005;16:535-548.
88. Weinstein BM. Vessels and nerves: marching to the same tune. *Cell.* 2005;120:299-302.
89. McGraw J, Gaudet AD, Oschipok LW, et al. Altered primary afferent anatomy and reduced thermal sensitivity in mice lacking galectin-1. *Pain.* 2005;114:7-18.
90. McGraw J, McPhail LT, Oschipok LW, et al. Galectin-1 in regenerating motoneurons. *Eur J Neurosci.* 2004;20:2872-2880.
91. Fukaya K, Hasegawa M, Mashitani T, et al. Oxidized galectin-1 stimulates the migration of Schwann cells from both proximal and distal stumps of transected nerves and promotes axonal regeneration after peripheral nerve injury. *J Neuropathol Exp Neurol.* 2003;62:162-172.
92. Pucho AC, Poirier F, Hair M, Bartlett PF, Key B. Role of galectin-1 in the developing mouse olfactory system. *Dev Biol.* 1996;179:274-287.
93. Rusciano D, Lorenzoni P, Burger MM. Specific growth stimulation in the absence of specific cellular adhesion in lung colonization by retinoic acid-treated F9 teratocarcinoma cells. *Int J Cancer.* 1992;52:471-477.
94. Pienta KJ, Naik H, Akhtar A, et al. Inhibition of spontaneous metastasis in a rat prostate cancer model by oral administration of modified citrus pectin. *J Natl Cancer Inst.* 1995;87:348-353.
95. Griffioen AW, van der Schaft DW, Barendsz-Janzen AF, et al. Anginex, a designed peptide that inhibits angiogenesis. *Biochem J.* 2001;354:233-242.
96. van der Schaft DW, Dings RP, de Lussanet QG, et al. The designer anti-angiogenic peptide anginex targets tumor endothelial cells and inhibits tumor growth in animal models. *FASEB J.* 2002;16:1991-1993.
97. Akerman ME, Pilch J, Peters D, Ruoslahti E. Angiostatic peptides use plasma fibronectin to home to angiogenic vasculature. *Proc Natl Acad Sci U S A.* 2005;102:2040-2045.
98. Brandwijk RJ, Nesmelova I, Dings RP, Mayo KH, Thijssen VL, Griffioen AW. Cloning an artificial gene encoding angiostatic anginex: from designed peptide to functional recombinant protein. *Biochem Biophys Res Commun.* 2005;333:1261-1268.
99. Pilch J, Franzin CM, Knowles LM, Ferrer FJ, Marassi FM, Ruoslahti E. The anti-angiogenic peptide anginex disrupts the cell membrane. *J Mol Biol.* 2006;356:876-885.
100. Mulder WJ, Strijkers GJ, Griffioen AW, et al. A liposomal system for contrast-enhanced magnetic resonance imaging of molecular targets. *Bioconjug Chem.* 2004;15:799-806.
101. Brandwijk RJ, Mulder WJ, Nicolay K, Mayo KH, Thijssen VL, Griffioen AW. Anginex-conjugated liposomes for targeting of angiogenic endothelial cells. *Bioconjug Chem.* 2007;18:785-790.
102. Andre S, Maljaars CE, Halkes KM, Gabius HJ, Kamerling JP. Discovery of galectin ligands in fully randomized combinatorial one-bead-one-compound (glyco)peptide libraries. *Bioorg Med Chem Lett.* 2007;17:793-798.