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REVIEW

Galectin-8: A matricellular lectin with key roles in angiogenesis

María F Troncoso[2], Fátima Ferragut[2], María L Bacigalupo[2], Víctor M Cárdenas Delgado[2], Lorena G Nugnes[2], Lucas Gentilini[3], Diego Laderach[3], Carlota Wolfenstein-Todel[2], Daniel Compagno[3], Gabriel A Rabinovich[3,4], and María T Elola[1,2]

[2]Instituto de Química y Fisicoquímica Biológicas Prof. Dr. Alejandro Paladini (UBA-CONICET), Facultad de Farmacia y Bioquímica; [3]Laboratorio de Glicómica Funcional, IQUIBICEN-CONICET, Departamento de Química Biológica. Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina; and [4]Laboratorio de Inmunopatología, Instituto de Biología y Medicina Experimental (IBYME – CONICET), Buenos Aires, Argentina

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Galectin-8 (gal-8) is a "*tandem-repeat*"-type galectin, containing two carbohydrate recognition domains connected by a linker peptide. gal-8 is expressed both in the cytoplasm and nucleus in vascular endothelial cells (ECs) from normal and tumor-associated blood vessels, and in lymphatic endothelial cells. Herein, we describe a novel role for gal-8 in the regulation of vascular and lymphatic angiogenesis and provide evidence of its critical implications in tumor biology. Functional assays revealed central roles for gal-8 in the control of capillary-tube formation, EC migration and in vivo angiogenesis. So far, two endothelial ligands have been described for gal-8, namely podoplanin in lymphatic vessels and CD166 (ALCAM, activated leukocyte cell adhesion molecule) in vascular ECs. Other related gal-8 functions are also summarized here, including cell adhesion and migration, which collectively demonstrate the multi-functionality of this complex lectin. Thus, gal-8 is an important component of the angiogenesis network, and an essential molecule in the extracellular matrix by providing molecular anchoring to this surrounding matrix. The implications of gal-8 in tumor angiogenesis remain to be further explored, but it is exciting to speculate that modulating gal-8-glycan interactions could be used to block lymphatic-vascular connections vital for metastasis.

Keywords: angiogenesis / CD166 / endothelium / galectin-8

Introduction

Galectin 8 (gal-8) is a member of the evolutionary conserved family of galectins, which share a high affinity for β-galactosides (Barondes et al. 1994). gal-8 contains two canonical *carbohydrate recognition domains* (CRDs) joined by a linker peptide (Bidon-Wagner and Le Pennec 2004; Zick et al. 2004). gal-8 was initially cloned from a rat liver cDNA library, and the resulting protein is composed of 316 amino acids (~35 kDa) with two CRDs. These N- and C-terminal domains share 35% homology and both contain sequence motifs conserved among most CRDs of galectins (Hadari et al. 1995). A human homolog gene, called *prostate carcinoma tumor antigen-1* (PCTA-1), was detected by screening a cDNA expression library of the human LNCaP cell line derived from advanced prostate cancer (Su et al. 1996; Gopalkrishnan et al. 2000). Concomitantly, another cDNA encoding for a human gal-8 was also isolated from a human brain hippocampus library, which shares 97% homology at the protein level with PCTA-1 (Hadari et al. 1997).

The human gal-8 gene (*LGALS8*) was shown to encode seven different isoforms, resulting from alternative splicing. The corresponding mRNAs encode for three isoforms that belong to the *tandem-repeat* galectin group (with two CRDs) and the others to the *prototype* group (possessing one CRD). The bi-CRD isoforms differ mainly in the size of their hinge region and have been called according to the length of this linker peptide as gal-8S (*small* gal-8, with a short linker region), gal-8M (*medium* gal-8, with an intermediate linker region) and gal-8L (*long* gal-8, with the longest linker peptide, Figure 1D). They are produced by alternative splicing at the level of the linker peptide and untranslated (3′ and 5′UTR) regions. In fact, three models of splicing have been proposed, which should be revised as they show some divergences (Figure 1; Gopalkrishnan et al. 2000; Bidon-Wagner and Le Pennec 2004; Ahmed et al. 2007). Of note, in the genomic structure of PCTA-1 (Figure 1C), the two exons at the extremities are present as partial or complete entities in the mature message due to internal processing (Gopalkrishnan et al. 2000). gal-8 isoforms that are prototype galectins, possessing only one CRD, have not been found at the protein level (Bidon et al. 2001; Bidon-Wagner and Le Pennec 2004; Ahmed et al. 2007). The amino acid sequence and CRD arrangement of human gal-8M (middle isoform with 317 amino acids; NP_963837; NM_201543) is shown in Figure 2; this isoform is a major naturally occurring variant of human gal-8, with a linker peptide comprising 28 amino acids. In addition, the functional consequences of gal-8 splicing are still poorly understood. Therefore,

[1]To whom correspondence should be addressed: Tel: +54-11-49648291; Fax: +54-11-49625457; e-mail: mtelola@gmail.com

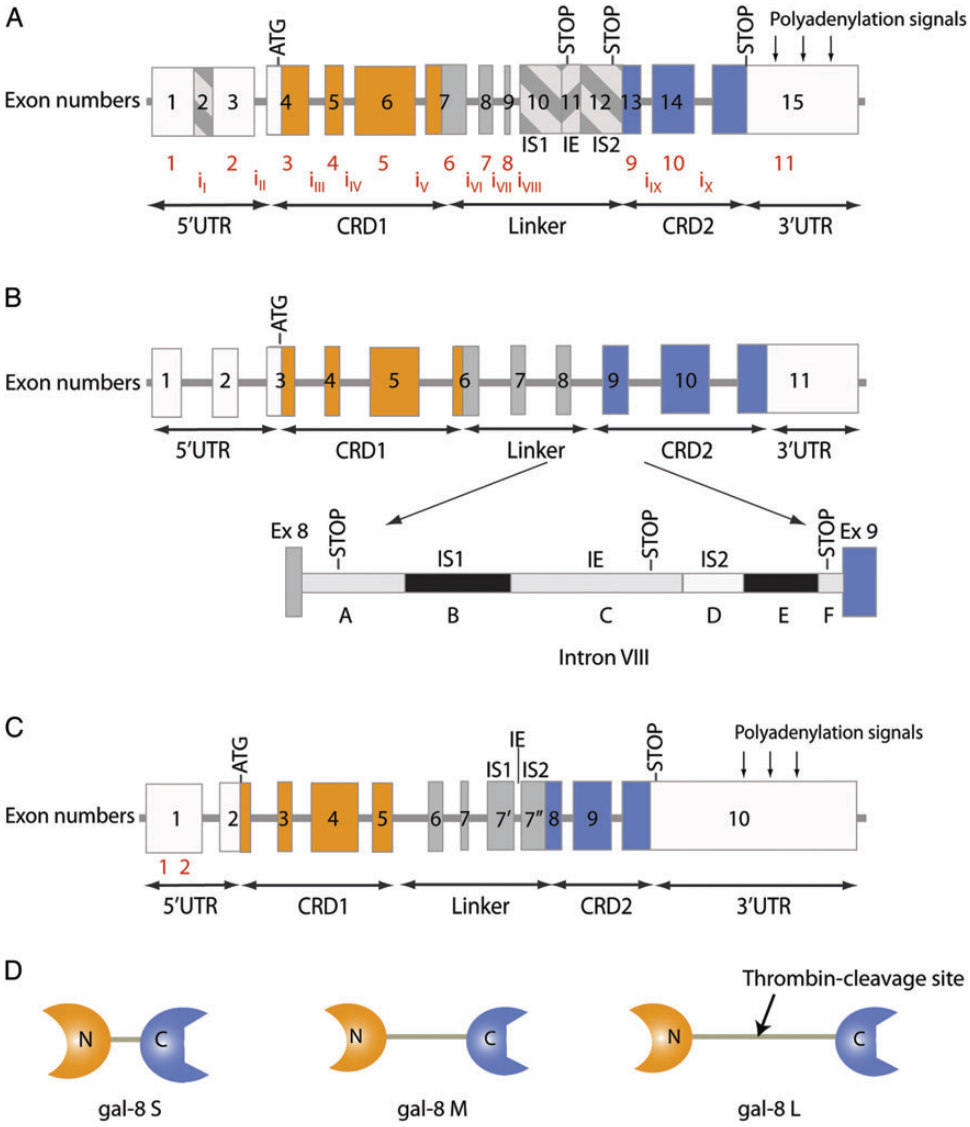

Fig. 1. Schematic representations of the genomic structure of gal-8, according to different models of alternative splicing, and the resulting protein isoforms. (**A**) Genomic structure showing coding and non-coding regions in both CDRs. Alternative exons are shown hatched. In the linker region, three alternatively spliced regions have been designated as insertional sequence 1 (IS1), intron IE (IE) and insertional sequence 2 (IS2) (Bidon et al. 2001; Bidon-Wagner and Le Pennec 2004). (**B**) Splicing occurs within intron VIII (A–F: alternatively spliced regions of intron VIII; Ahmed et al. 2007). Numbering of exons and/or introns in model B is also shown below the boxes in models A and C for comparison. (**C**) The two exons at the extremities in PCTA-1 genomic structure are present as partial or complete entities in the mature message due to internal processing; different polyadenylation signals have been detected (Gopalkrishnan et al. 2000). (**D**) gal-8 protein isoforms detected by western blot in different tissues and cell lines; the thrombin-cleavage site is indicated in the linker peptide of gal-8L (Nishi et al. 2006). UTR, untranslated region; Ex, exon; i, intron.

potential biological effects of specific gal-8 splice variants in EC biology and angiogenesis, which have been demonstrated for gal-9 (Heusschen et al. 2014), should be evaluated in the future.

Regarding carbohydrate specificity, both gal-8 CRDs differ dramatically (Hirabayashi et al. 2002; Ideo et al. 2003; Patnaik et al. 2006; Carlsson, Oberg, et al. 2007; Stowell et al. 2008), and both ones are required for different effects, in addition to the proper length of the linker peptide (Levy et al. 2006). The N-terminal domain of gal-8 (gal-8N) has much higher affinity for 3′-*O*-sialylated-lactose and 3′-*O*-sulfated-lactose than for oligosaccharides terminating in Galβ1-3/4GlcNAc. The crystal structure of gal-8N or gal-8Null (a mutant bi-CRD form of human gal-8 without linker peptide) in complexes with lactose and Neu5Acα2-3Galβ1-4Glc (α2-3-sialyl-lactose) revealed that Arg^{45}, Gln^{47} and Arg^{59} residues, which are conserved in the N-CDR of rat gal-8 and in the N-CRD of all mouse and human gal-8 isoforms (Tribulatti et al. 2007; Ideo et al. 2011), interact with the sialic acid or sulfate moieties of 3′-sialyl- and 3′-sulfo-lactose. Arg^{45} is also conserved in other tandem-repeat galectins: in human gal-9 N- and C-CRD, which have significant affinity for α2-3-sialylated oligosaccharides (Yoshida et al. 2010), and in human gal-4 N-CRD, which binds sulfated glycans (Ideo et al. 2007, 2011). Amino acids responsible for

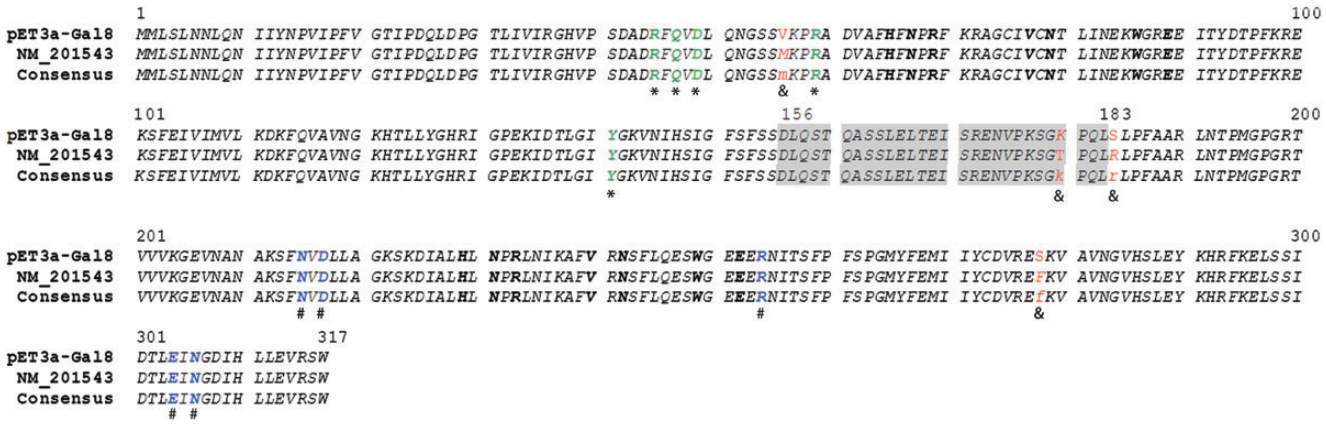

Fig. 2. Protein sequence alignments of the human gal-8M isoform (gal-8M). The sequence cloned in the expression vector pET-3a-*gal8* (Delgado et al. 2011) is compared with human gal-8M (GenBank: NM_201543; NP_963837), a middle isoform of gal-8: the four different amino acids are marked with &. Conserved amino acids between galectins are shown in bold. Amino acids which play key roles in glycan interactions are marked with * for gal-8N and are indicated with # for gal-8C. The linker peptide is highlighted in gray.

strong binding to α2-3 sialylated-lactose are absent in the gal-8C domain (Ideo et al. 2011; Yoshida et al. 2012; Kumar et al. 2013).

In fact, gal-8 binding to the cell surface does not require its fine specificity (Patnaik et al. 2006), but it appears to be mediated by the combined interaction of the two CRDs with "second best" ligands of moderate affinity (Carlsson, Oberg, et al. 2007). In intact bi-CRD isoforms of gal-8, the two domains act independently of each other in solution, whereas at a surface they act together. Thus, gal-8 binding and signaling at cell surfaces can be explained by combined binding of the two CRDs to low- or medium-affinity ligands, and their highest affinity ligands, such as sialylated galactosides, are probably not required. In conclusion, gal-8 functions require cooperative interactions between its two CRDs (Carlsson, Oberg, et al. 2007). Of note, a role for gal-8 fine specificity in intracellular targeting has been found: the binding of gal-8 N-CRD to sialylated galactosides can determine its intracellular targeting (Carlsson, Carlsson, et al. 2007). Some sialoglycoproteins recycle between the plasma membrane and Golgi via an endocytic pathway (Slimane et al. 2000; Potter et al. 2006), and their interaction with the gal-8 N-CRD might mediate this process. Thus, gal-8 could be a "driver" (similar to gal-3 and -4; Delacour et al. 2005, 2006) or a "passenger" in such intracellular targeting (Carlsson, Carlsson, et al. 2007). In this sense, a role for gal-8 in promoting coagulation factor V (FV) endocytosis in megakaryocytes has also been described: FV internalization is effectively inhibited by an antibody directed against gal-8, and the reduced expression of gal-8 by siRNA technology impairs FV uptake (Zappelli et al. 2012).

Galectin-8 in endothelial cells and serum

To gain insight into the possible function of galectins in endothelial cell (EC) biology, Thijssen et al. (2008) analyzed the expression of all known human galectins in the endothelium. Particularly, they determined that gal-8 mRNA is expressed in quiescent human umbilical vein endothelial cells (HUVECs) and human microvascular endothelial cell (HMEC) lines (RF24 and EVLc2). Also, they confirmed expression at the protein level in HUVECs by western blotting, showing three gal-8 bands of 34, 36 and 38 kDa. By immunohistochemistry, the authors demonstrated a dot-like cytoplasmic staining pattern. In vivo endothelial expression of gal-8 was observed in colon and kidney but was undetectable in placenta and liver vessels. Interestingly, EC activation in vitro, by culturing cells under high serum concentrations, significantly decreased intracellular gal-8 expression while it increased the expression of the membrane-bound protein. An altered localization was also observed in vivo; in normal tissues, gal-8 was found in the cytoplasm but mainly detectable in the nuclei of ECs. However, in human colon carcinoma tissues, the number of gal-8-positive ECs decreased, being this lectin exclusively detected in the nucleus (Thijssen et al. 2008, 2013).

Interestingly, we also found the presence of three gal-8 isoforms (34: gal-8S, 36: gal-8M and 38 kDa: gal-8L; Figure 1D) in vascular bovine aortic endothelial cells (BAECs) by western blot. We analyzed gal-8 intracellular localization in BAECs by immunofluorescence, detecting both cytoplasmic and nuclear distribution of this lectin. To study the in vivo expression and localization of gal-8 in blood vessels, we performed immunohistochemical studies in paraffin-embedded sections of human prostate and breast normal versus tumor tissues. Blood vessels contiguous to normal or tumor prostate tissues showed both nuclear and cytoplasmatic localization of this lectin. Blood vessels in breast tissues showed strong nuclear staining and diffuse cytoplasmic reactivity. In conclusion, gal-8 is localized both in cytoplasmic and nuclear compartments of normal and tumor-associated ECs from human prostate and breast tissues (Delgado et al. 2011). In addition, gal-8 has been found to be highly expressed in human primary dermal lymphatic endothelial cells (LECs), mainly in the cytoplasm. gal-8 mRNA and protein levels were significantly higher in LECs than in human primary dermal vascular endothelial cells (BECs); moreover, two bands (isoforms) reactive with anti-gal-8 antibody were detected in LECs and BECs by western blot (Cueni and Detmar

2009). Collectively, these results suggest that gal-8 expression and localization control the biology of blood and lymphatic vessels.

In serum, gal-8 levels were analyzed in patients carrying different tumors versus healthy individuals. Serum gal-8 concentrations were 1.8-fold higher in patients with colorectal cancer and 5.6 higher in those with metastases with respect to healthy individuals, as measured by ELISA ($n = 51$). Similarly, the concentrations of gal-8 were 1.8-fold higher in sera from patients with breast cancer as compared with healthy individuals ($n = 40$; Barrow et al. 2011).

Galectin-8 role in vascular angiogenesis

Vascular angiogenesis is a fundamental process by which new blood vessels sprout from pre-existing ones, a process that is controlled by a relative balance of inducers and inhibitors of angiogenesis (Folkman and Shing 1992; Folkman 2003). We demonstrated that recombinant gal-8, when added to Matrigel, induced the formation of extensive capillary networks in BAECs from the macrovasculature. In terms of tube length, gal-8 promoted the formation of 3-fold longer tubules as compared with those observed in negative controls. This effect relied on protein–glycan interactions as it was prevented by specific disaccharide inhibitors such as lactose and thiodigalactoside (TDG), but not by the unrelated saccharide sucrose. gal-8-embedded Matrigel also induced the formation of widespread tubular networks by HMECs, demonstrating that blood vessels belonging to the micro- or macrovasculature can be stimulated by gal-8. When gal-8 was silenced by a specific siRNA, a reduction in tube length relative to non-silenced cells was observed after transfection, showing that the endogenous gal-8 also promoted tubule formation, probably via its secretion to the Matrigel (Delgado et al. 2011). We also performed in vivo angiogenesis experiments with Matrigel containing recombinant gal-8, vascular endothelial growth factor (VEGF) or vehicle alone injected in mice. Those plugs containing either VEGF or recombinant gal-8 showed increased number of ECs as compared with control Matrigel plugs. Thus, similar to VEGF, gal-8 promotes angiogenesis in vivo in a dose-dependent manner. Importantly, in contrast to gal-1, gal-8 was not found to be up-regulated in vivo in response to anti-VEGF treatment or following exposure to hypoxic conditions (Croci et al. 2012, 2014). Therefore, galectins can modulate EC biology and angiogenesis through different mechanisms. Based on the evidence summarized in the previous section that describes gal-8 expression in ECs, it can be hypothesized that under angiogenic stimulatory conditions, gal-8 expression levels in these cells seem to remain unaltered, but further studies are needed to elucidate the precise mechanisms that control gal-8 expression in the endothelium.

Putative role of galectin-8 in lymphangiogenesis

Lymphangiogenesis, the process of new lymphatic vessel formation from pre-existing vessels, is used by normal tissues for development and repair of lymphatic vasculature, whereas tumors rely on this process for the establishment of lymphatic-vascular connections that are so vital for metastasis (Pepper and Skobe 2003; Cueni and Detmar 2006; Ji 2006). gal-8 (but not gal-1, -2, -3 and -7) was found to specifically interact with podoplanin (PDPN), a key molecule in LECs, in a human protein microarray probed with a fusion protein of PDPN ectodomain (PDPN-Fc). gal-8 bound to glycosylated PDPN-Fc expressed by human embryonic kidney HEK293 cells, but did not associate with unglycosylated PDPN-Fc derived from CHO ldlD cells, which are not able to produce *O*-linked carbohydrate chains, demonstrating that interactions were glycan-dependent (Cueni and Detmar 2009). LEC-derived PDPN directly interacted with gal-8 when co-immunoprecipitation was performed in LEC lysates. Furthermore, gal-8 was found to be highly expressed by LECs, being involved in promoting LEC adhesion and migration. Although gal-8 promoted adhesion and migration of LECs, it inhibited the formation of tubular-like structures by LECs when incorporated into a collagen matrix (Cueni and Detmar 2009; Figure 3).

Other related functions of galectin-8

Cell adhesion

gal-8 behaves as an extracellular matrix (ECM) protein that positively or negatively regulates cell adhesion, depending on the extracellular context as well as on cell surface counter-receptors like integrins (Levy et al. 2001; Zick et al. 2004; Elola et al. 2007). In the case of human LECs, they firmly adhered and spread on gal-8 after seeding; however, when added to the cell culture medium in a soluble form, gal-8 almost completely prevented the adhesion of LECs to any substrate, due to cell aggregation (Cueni and Detmar 2009). In human ECV304 ECs, adhesion to immobilized gal-8 produced the initial formation of lamellipodia followed by the appearance of F-actin microspikes, a phenomenon also observed in CHO-P and NIH-hIR cells. Moreover, ECV304 cells spread fast on gal-8, and wortmannin, a phosphatidylinositol-3-kinase (PI3K) inhibitor, diminished gal-8-induced cell spreading, although this agent did not affect the spreading on fibronectin. Therefore, downstream effectors of PI3K selectively regulate cytoskeletal rearrangements that occur when ECV304 cells adhere to and spread on gal-8 (Levy et al. 2003).

Regarding cancer cell adhesion to the vascular endothelium, as circulating gal-8 levels are increased in sera from patients with colon and breast cancer, the effects of this lectin on cell adhesion were carefully analyzed (Barrow et al. 2011). Pre-incubation of human colon cancer HT29-5F7 cells (a clone of HT29 cells with resistance to 5-fluorouracil) with recombinant gal-8 induced a dose-dependent increase of colon cell adhesion to HUVECs, which was inhibited by lactose. gal-8 also induced increased adhesion of HT29-5F7 and SW620 cells to human microvascular lung endothelial cells (HMVECs-L), but not in the case of HT29 cells. Thus, increased circulating levels of gal-8 might contribute to promote metastasis. Moreover, gal-8 induced adhesion to vascular endothelium of HBL-100 human breast epithelial cells transfected with the Thomsen-Friedenreich (Galβ1-3GalNAc) disaccharide expressed by the cancer-associated transmembrane mucin protein 1 (MUC1). Thus, gal-8 might promote dissemination of certain tumor cells by facilitating adhesion to blood vascular endothelium, at least in part via mechanisms involving Thomsen-Friedenreich/MUC1. Knocking down MUC1 using a

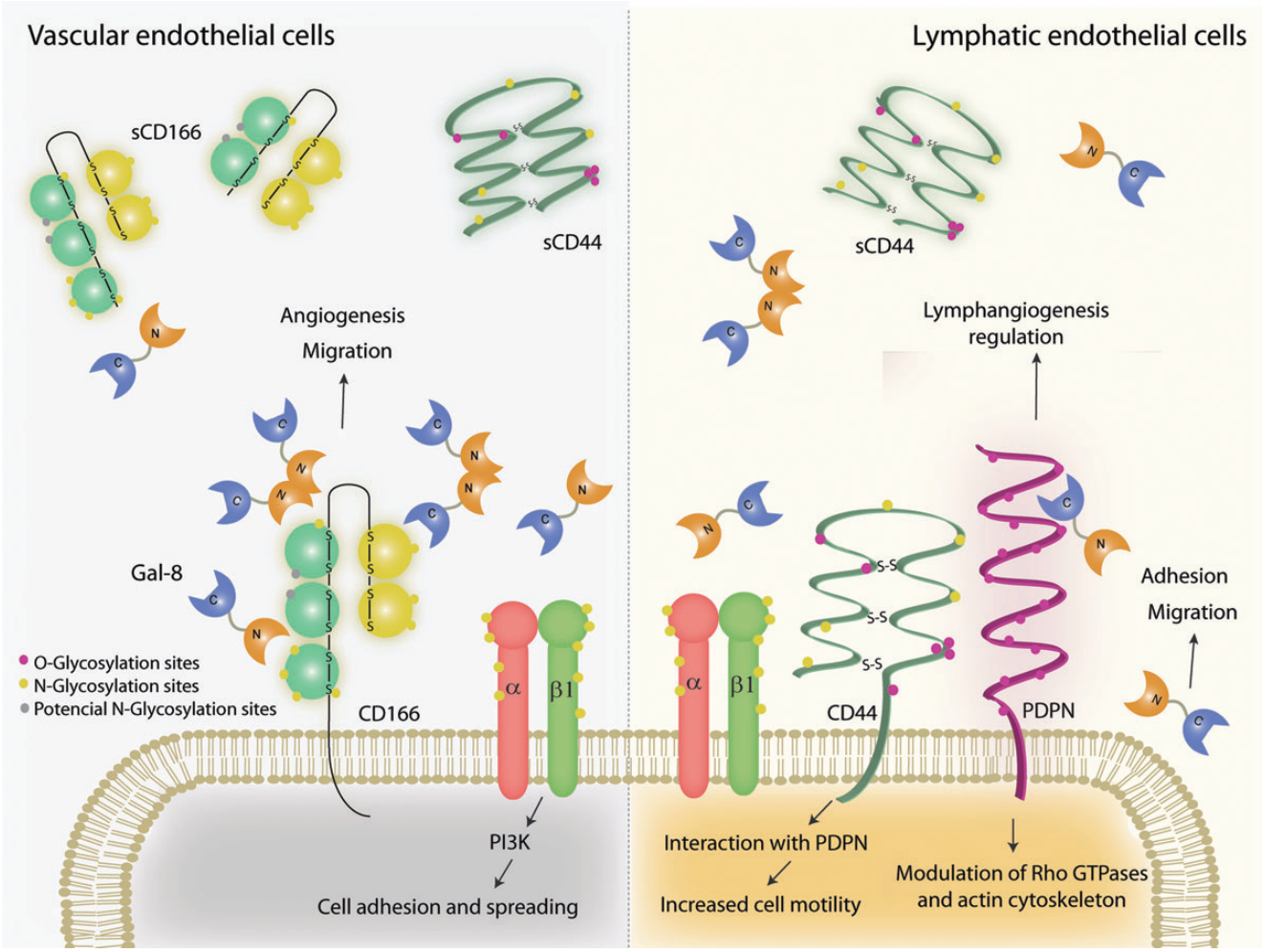

Fig. 3. Glycan-dependent interactions of gal-8 in endothelial cells and their microenvironment. gal-8 can act as a dimer or monomer. It interacts with CD166 in vascular ECs and promotes angiogenesis and migration. In lymphatic ECs, gal-8 promotes adhesion and migration and regulates lymphangiogenesis. PDPN *per se* promotes lymphangiogenesis and interacts with a variety of ligands, such as CD44, inducing cytoskeleton rearrangements. CD166 and CD44 also possess soluble forms (sCD166 and sCD44, respectively).

specific siRNA partially abolished gal-8-mediated cell adhesion to HMVECs-L (Barrow et al. 2011). Furthermore, gal-8 has been suggested as a metastasis-associated molecule in an ECM microarray and in vitro adhesion experiments in cells from murine lung adenocarcinoma. Combinations of fibronectin and gal-8 promoted cell adhesion profiles that allowed the discrimination of metastatic cell populations from primary tumor cells (Reticker-Flynn et al. 2012).

Other tandem-repeat galectins also regulate cell adhesion to the vascular endothelium. Thus, overexpression of gal-9M or gal-9S isoforms induces up-regulation of E-selectin in LoVo colon tumor cell and adhesion to HUVECs. Interestingly, gal-9L overexpression induces down-regulation of E-selectin expression and it does not result in an increased cell adhesion to ECs, although it produces an increased adhesion of these cells to Matrigel (Zhang et al. 2009). This further illustrates that the effects of a galectin on adhesion depend on which ligands are available in a given setting (Heusschen et al. 2013). gal-9 was also shown to suppress both attachment and invasion of different tumor cells in vivo by inhibiting the binding of adhesive molecules on tumors to ligands of both vascular endothelium and ECM, respectively, resulting in the suppression of metastasis (Nobumoto et al. 2008).

Cell migration

Regarding EC migration, we demonstrated that immobilized gal-8 induced the migration of serum-starved ECs in a dose-dependent fashion. Indeed, ECs that had not been serum-starved could be stimulated by lower concentrations of gal-8 when cultured in the absence of serum during the migration period. This pro-migratory effect of gal-8 could be partially blocked by specific sugars such as lactose and TDG. Thus, in addition to promoting capillary-tube formation and in vivo angiogenesis, gal-8 promotes vascular EC migration in a dose- and glycan-dependent fashion. Next, we performed migration assays in the presence or absence of anti-CD166 antibodies: gal-8 promoted BAEC migration as compared with controls, and anti-CD166 blocked gal-8 effects on EC migration (Delgado et al. 2011). gal-8 has been involved in promoting primary

human dermal LEC migration. Thus, in transwell migration assays, gal-8 significantly enhanced haptotactic migration of LECs to the lower chamber, when compared with other unrelated proteins such as albumin (Cueni and Detmar 2009).

Galectin-8 ligands in ECs

Podoplanin

PDPN is a sialomucin-like transmembrane protein highly expressed in lymphatic, but not blood vascular cells, which has emerged as a potential gal-8-binding partner (Cueni and Detmar 2009). PDPN has a wide variety of functions including regulation of organ development, cell motility, tumorigenesis and metastasis (Wicki and Christofori 2007; Astarita et al. 2012). It contributes to polarization and migration of LECs (Navarro et al. 2011). Some of its interactions with other proteins such as the C-type lectin CLEC-2 (Kato et al. 2008) or gal-8 (Cueni and Detmar 2009) depend on the carbohydrate moieties present in its ectodomain (Figure 3). In fact, the existence of di-sialylated core 1 sugar moieties attached to O-glycosylation sites in PDPN has been previously reported (Kaneko et al. 2007). Interestingly, PDPN is required for CLEC-2-dependent platelet aggregation, but it also seems to have an intrinsic effect on proliferation or differentiation (Astarita et al. 2012). Moreover, it has been reported that overexpression of PDPN promoted breast cancer cell motility in vitro and lymphangiogenesis in breast carcinoma xenografts (without affecting primary tumor growth), which altogether would potentiate metastasis to lymph nodes (Cueni et al. 2010).

CD166

CD166 is a transmembrane glycoprotein belonging to the immunoglobulin superfamily, which is involved in homo- and heterophilic cell interactions and trans-EC migration (Swart et al. 2005; Weidle et al. 2010). The whole transmembrane protein CD166 has been reported to interact with itself, with sCD166 (Ikeda and Quertermous 2004) and with CD6 (Bowen et al. 1995; Patel et al. 1995). CD166 has been identified as a glycoprotein composed of β1-6 branched oligosaccharides (Ochwat et al. 2004) and eight N-glycosylation sites (Weidle et al. 2010). Interestingly, CD166 was found in several cell types, including pulmonary microvascular ECs (PMVECs) (Masedunskas et al. 2006; Ofori-Acquah et al. 2008), HUVECs, human aortic ECs, human coronary artery ECs and HMVECs-L (Ikeda and Quertermous 2004). It has been implicated in the modulation of tube formation in embryonic and adult ECs (Ohneda et al. 2001; Ikeda and Quertermous 2004). In HMVECs, soluble isoforms of CD166 (sCD166, sALCAM), containing the single amino terminal Ig-like domain of CD166, were reported. sCD166 significantly enhanced migration of HMVECs, whereas it inhibited tube formation on Matrigel; sCD166 can partially inhibit CD166–CD166 homophilic interactions due to its ability to bind to CD166 (Ikeda and Quertermous 2004). We previously identified CD166 (ALCAM, Swiss-Prot accession #Q9BH13) as a gal-8 ligand from BAEC lysates, by mass spectrometry. To confirm whether gal-8 interacts with endogenous CD166, we performed co-immunoprecipitation experiments using recombinant gal-8 and BAEC lysates, demonstrating their direct association. To evaluate whether CD166 could mediate gal-8-induced angiogenesis, we carried out tubulogenesis experiments, preincubating BAECs with or without different anti-CD166 antibodies: anti-CD166 antibodies significantly diminished BAEC capillary formation induced by gal-8. Thus, CD166, a gal-8 ligand in BAECs, can mediate at least in part the effect of this lectin on tubulogenesis (Delgado et al. 2011). We also confirmed the direct interaction between CD166 and gal-8 by surface plasmon resonance, with an apparent dissociation constant of $K_d = 2 \times 10^{-6}$ M, which indicated a specific binding that could be blocked by β-galactoside carbohydrates. We also observed that CD166 was localized both at the cell surface and in the cytoplasm of BAECs as revealed by flow cytometry. Moreover, in functional assays, CD166 was demonstrated to mediate at least part of the pro-angiogenic and pro-migratory effects triggered by gal-8 in BAECs (Delgado et al. 2011; Figure 3).

Integrins

gal-8 functions as a matrix-associated protein, whose interactions with integrins mediate, at least in part, both its adhesive and anti-adhesive properties (Levy et al. 2001, 2003). gal-8 binds to a variety of integrins such as $\alpha_3\beta_1$, $\alpha_6\beta_1$, α_M and α_5, depending on the cell types (Hadari et al. 2000; Levy et al. 2003; Nishi et al. 2006). In human vascular ECs, integrins α_3 and β_1, and to a lesser extent α_4, have been identified as potential ligands on immobilized gal-8: quantitative densitometry revealed that gal-8 bound 26 and 18% of the total α_3 and β_1 integrins, respectively (Hadari et al. 2000). In LEC lysates, endogenous β_1 integrin was pulled-down adding recombinant gal-8, revealing the direct interaction between both molecules (Cueni and Detmar 2009; Figure 3).

CD44

CD44 is a multifunctional receptor with extensive N- and O-glycosylation (Brazil et al. 2013) involved in numerous cell–cell and cell–ECM interactions, which influences cellular processes including adhesion, migration, invasion and survival (Ponta et al. 2003; Orian-Rousseau 2010). PDPN interacts with CD44, and this association regulates lamellipodia extension and promotes directional motility (Martín-Villar et al. 2010). A specific CD44 variant designated CD44vRA, which was found in the synovial fluid from rheumatoid arthritis patients, has been described as a gal-8 ligand. Using surface plasmon resonance, the specific binding of different CD44 isoforms to immobilized gal-8 was analyzed, detecting that the binding affinity of CD44vRA was the highest ($K_d = 5.8 \times 10^{-9}$ M), and it was 5- and 170-fold greater than that of CD44v3-10 ($K_d = 2.7 \times 10^{-8}$ M) and sCD44 ($K_d = 10^{-6}$ M), respectively. Furthermore, gal-1 and gal-3 exhibited only negligible binding to CD44. These results suggest that CD44vRA serves as a high-affinity receptor for gal-8 (Eshkar-Sebban et al. 2007). The existence of gal-8-mediated interactions between CD44 and PDPN cannot be ruled out and should be further investigated.

Conclusion

gal-8 is an angiogenesis modulator in vascular and lymphatic endothelium, which recognizes different ligands in both types

of endothelia. In vascular ECs from the micro- and macrovasculature, we demonstrated that gal-8 promotes angiogenesis. We suggest that CD166 interacts with gal-8 in the vascular endothelium, and at least partially regulates gal-8-mediated angiogenesis and migration. Accordingly, CD166 has been implicated in migration, trans-EC migration, and in homotypic/homophilic, and heterotypic/heterophilic adhesion in different models. In LECs, gal-8 promotes cell adhesion and migration when immobilized onto a surface, but inhibits the formation of tube-like structures when incorporated into a collagen matrix. PDPN is a key modulator of the multi-step process by which the lymphatic vasculature assumes its definitive shape, and it is a novel glycosylation-dependent partner of gal-8. PDPN by itself can promote in vivo lymphangiogenesis, and it might contribute to regulate the effects of gal-8 on LEC adhesion, migration and lymphangiogenesis. Other essential gal-8 ligands such as β_1 integrins from vascular or lymphatic ECs complete the scenario. Additional studies will be required to address the relevance of gal-8/CD166 and gal-8/PDPN interactions in regulating normal and/or pathological vascular or lymphatic angiogenesis and the signaling mechanisms underlying these effects.

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Conflict of interest

None declared.

Abbreviations

BAECs, bovine aortic endothelial cells; BECs, human primary dermal vascular endothelial cells; CRD, carbohydrate recognition domain; ECs, endothelial cells; ECM, extracellular matrix; gal-8, galectin-8; gal-8S, *small* gal-8; gal-8M, *medium* gal-8; gal-8L, *long* gal-8; gal-8C, C-terminal domain of gal-8 or C-CRD; gal-8N, N-terminal domain of gal-8 or N-CRD; HMECs, human microvascular endothelial cells; HMVECs-L, human microvascular lung endothelial cells; LECs, human primary dermal lymphatic endothelial cells; PDPN, podoplanin; PCTA-1, prostate carcinoma tumor antigen-1 (gal-8); TDG, thiodigalactoside; VEGF, vascular endothelial growth factor.

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