Molecular Mechanisms Underlying the Role of Galectin-8 as a Regulator of Cancer Growth and Metastasis

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Abstract

Galectin-8 (Gal-8) is a member of the galectin family of animal lectins that regulate a myriad of biological processes including cell growth, cell transformation, embryogenesis, apoptosis, cell adhesion and immune responses. Gal-8 expression increases in several, though not all, cancerous tissues including lung, bladder, kidney, prostate, and breast tissues. Based on its prevalence, an estimated ~500,000 newly diagnosed cancer patients/year are expected to possess an amplified Gal-8 gene. Yet, the molecular mechanisms underlying its role in cancer growth and metastasis remain incompletely understood. Here we describe potential modes of action of Gal-8 that might account for its central role in cancer biology. The evidence, gathered thus far, implicates Gal-8 as a driver of a ‘vicious cycle,’ whereby cancer cells that overexpress and secrete Gal-8, benefit from its potential to promote their own growth; potentiate epithelial mesenchymal transition, and induce secretion of metastasis-promoting agents at the metastatic niche that induce further recruitment and seeding of cancer cells. Further in-depth studies related to its mode of action, are expected to support ongoing efforts aimed at implementing Gal-8-targeted therapies for the treatment of cancer patients.

A. Introduction

Galectin-8 (Gal-8) (1) is a member of the galectin family of animal lectins (2) that regulate a myriad of biological processes including cell growth, cell transformation, embryogenesis, apoptosis, cell adhesion and immune responses [reviewed in (3–7)]. It is a 34kDa protein, made of two carbohydrate recognition domains (CRDs) of about 140 amino acid each, joined by a linker peptide of various lengths. The two CRDs share ~40% sequence identity and exhibit differential glycan binding specificities (1, 8, 9). The N-terminal CRD (Gal-8N), has unique sugar binding residues that recognize a broader spectrum of glycans compared to the C-terminal CRD (Gal-8C), and exhibits preferential binding towards anionic sugars including 3’-O-sulfate/3’-O-sialylated lactose (10). Recent crystallographic study highlighted preference of Gal-8N towards recognizing neolacto-series over lacto-series glycosphingolipids implying specific roles for Gal-8 over other galectins (11). While lacking a signal peptide suitable for ER/Golgi-mediated secretion, Gal-8, like other galectins, is externalized by an atypical secretory mechanism (12). Atypical secretion (13–15) is not a unique property of galectins, because a number of other cytoplasmic proteins like thioredoxin (16), IL-1β (17) and basic FGF (18) lack a signal sequence, yet are externalized and function extracellularly.

Gal-8 is widely expressed in a large number of tissues including lung, liver, kidney, spleen, hind limb, and cardiac muscle (1, 19, 20). Low levels of expression were detected in intestine, colon, fat, and thymus and almost no expression was detected in hematopoietic cells. These results indicate that although Gal-8 is a fairly abundant protein, it is not ubiquitously expressed. Gal-8 is a cytoplasmic protein. However, similar to other galectins (21–23), it is not uniformly spread within the cell. Instead, it shows a micro-clustering pattern reminiscent of that seen with proteins associated with mitochondria, the Golgi or trans-Golgi membranes (1, 20, 24–26). Nuclear expression of Gal-8 is evident as well (27). Expression of Gal-8 seems to be developmentally regulated. Very low levels of expression were noted in whole embryos (1), while high levels of expression were noted in adult tissues. Hence, Gal-8, like other galectins (28–31), can be implicated as a regulator of cell growth, embryogenesis and development.

Upon secretion, Gal-8 acts as an extracellular matrix protein equipotent to fibronectin in promoting cell adhesion by ligation and clustering a selective subset of cell surface integrins and CD44 (19, 32, 33). This allows local signals emitted by secreted Gal-8 to specify territories available for cell adhesion and migration (19, 33–35). Aside from its role as an extracellular regulator of cell functions, Gal-8, like other members of the galectin family (5), has intracellular functions. In particular, Gal-8-mediated recognition of intracellular vesicles, damaged following bacterial infection, targets these vesicles for autophagy, thus providing a unique form of intracellular antimicrobial defense elicited by Gal-8 (36). Altogether, the biological effects of Gal-8 resemble the mode of action of other galectins in a number of aspects; nonetheless, several features of Gal-8 single it out from the entire galectin family. These unique features, mainly its role as a regulator of cancer growth and
metastasis, are the subject of the present review.

B. Gal-8 and Cancer

Galectins emerge as key players in the regulation of cancer growth and metastasis. They contribute to many hallmarks of cancer, including sustained cell proliferation, inhibition of apoptosis, evasion of immune surveillance, promotion of angiogenesis, and induction of the metastatic process (37–40). They do so by modulating the interactions between tumor, endothelial, stromal, and immune cells (37–42). Yet, in spite of the wealth of information concerning the expression of different galectins during tumor progression (37), the molecular basis for their role in cancer growth and metastasis remains incompletely understood.

Gal-8 expression increases in several, though not all, cancerous tissues including lung, bladder, kidney, prostate, and breast tissues (27, 43, 44). It tightly controls the migratory and metastatic properties of tumor cells (45) and it is associated with poor prognosis in a number of cancer types (43). Several studies reported selective amplification of the Gal-8 gene (LGALS8) in human tumors of breast (14–31% of samples examined); prostate (4–25%) and lung (6–7%) (24, 27, 43, 46). Based on its prevalence, an estimated ~500,000 newly diagnosed cancer patients/year are expected to possess an amplified Gal-8 gene (Fig. 1). The level of amplification of the Gal-8 gene in different cancer patients exceeds by far that of other common galectins like Gal-1 and Gal-3 (Fig. 2), thus attesting as to the central role, played by Gal-8 in the regulation of cancer, among members of the galectin family.

Gal-8 has been identified as one of the five most frequently expressed tumor antigens in a mouse model of HER2-neu-positive and estrogen receptor (ER)-negative breast cancer (47). It has been considered as a mediator of hereditary prostate cancer (48) and was named ‘prostate cancer tumor antigen-1’ (24). Gal-8 levels are increased in sera from patients with colon and breast cancer, in which it was shown to increase adhesion of cancer cells to human microvascular lung endothelial cells. Thus, increased circulating levels of Gal-8 might promote metastasis (49, 50). Indeed, the frequency of lymph node metastasis is completely abolished upon silencing of Gal-8 in a mouse model of prostate cancer (45). Similarly, binding of lung cancer cells to Gal-8 in combination with fibronectin is strongly associated with metastatic progression of lung adenocarcinoma (51). Still, a number of studies reported on decreased expression of Gal-8 in association with favorable early tumor progression (52, 53). Low Gal-8 expression indicated poor overall and disease-free survival of patients with non-metastatic gastric cancer (54), suggesting that the role of Gal-8 in cancer

Fig. 1. Expected amplification of the LGALS8 gene in cancer patients. The Gal-8 gene is expected to be amplified in about ~500,000 newly diagnosed cancer patients/year. The scheme shows the distribution of cancer types among these patients. Data is taken from: www.cbioportal.org.

Fig. 2. Amplification of the LGALS8 gene. The Gal-8 gene is amplified to a much higher extent than Gal-3 or Gal-1 in a wide variety of human tumors. Dashed line is placed at the 5% alteration frequency. Data is taken from 164 studies: www.cbioportal.org.
growth and metastasis is more complex than initially suggested. Given that so far, relatively few studies analyzed Gal-8 expression in cancer patients, more conclusive evidence awaits additional works in larger patient groups.

C. Molecular Mechanisms Underlying the Mode of Action of Gal-8 as an Inducer of Cancer Growth and Metastasis

Building upon our current knowledge, at least three major mechanisms can be implicated as mediating the effects of Gal-8 on cancer growth and metastasis: i. Promotion of cell-matrix interactions that increase cell growth, adhesion, and selective metastatic seeding; ii. Induction of cancer cell proliferation and stimulation of epithelial-mesenchimal transition (EMT) by autocrine and paracrine mechanisms; iii. Induction in target cells the expression and secretion of cytokines and chemokines that promote by chemotraction, recruitment of cancer cells to the metastatic niche.

i. Modulation of cell-matrix interactions

Tumor microenvironment dramatically impacts tumor growth, adhesion, and metastatic progression (55). Changes in cancer cell-extracellular matrix (ECM) interactions can influence each stage of the metastatic cascade, from the loss of basement membrane adhesion to colonization at distant sites (56). Cell-matrix interactions depend to a large extent upon the engagement of specific ligands by a diverse class of cell surface $\alpha\beta$ heterodimeric integrins (57–59), that mediate many cell functions including cell adhesion, migration, and invasion (60). Integrins also have a multitude of intracellular effects both on cytoskeletal organization, and networks of signaling pathways (58, 61–63).

Acting as a matricellular protein, capable of binding both ECM proteins and cell adhesion molecules (34), Gal-8 can positively or negatively affect cell adhesion, depending on the extracellular context (19, 33, 64). As such, it can modulate cell growth and selective metastatic seeding. When immobilized onto matrix, Gal-8 acts as an ECM protein equipotent to fibronectin in promoting cell adhesion, spreading and migration (33). Accordingly, silencing of Gal-8 inhibits filopodia formation, migration, and homotypic aggregation of cancer cells (45), processes that are actively engaged in metastatic progression.

Cell adhesion to Gal-8 involves its interaction with a subset of integrins including $\alpha_1$, $\alpha_3$, $\alpha_6$, and $\beta_1$ while it interacts to a limited extent with $\alpha_4$ and $\beta_3$ integrins (19). Complex formation between Gal-8 and integrins triggers integrin-mediated signaling cascades such as Tyr phosphorylation of FAK and paxillin, and a robust and sustained activation of the ERK and PI3K signaling pathways that promote cell growth and metastasis (33, 65). In particular, Gal-8 receptors $\alpha_\beta_1$ and $\alpha_\beta_3$ integrins (19), expressed by breast (66), prostate (67) and lung metastatic cells (51), bind to Gal-8/fibronectin complexes preferentially at the metastatic niche (51), thus promoting metastatic seeding. Importantly, the Gal-8/fibronectin complexes are not produced solely by the tumor cells, but also by the naïve cells present in these tissues (51). Of interest, $\alpha_\beta_1$ integrin, the major dimer that interacts with Gal-8 (19), seems to be a major player in the process of adhesion and seeding of metastatic cells (51).

Aside from integrins, Gal-8 interacts with other proteins involved in cell adhesion. For example, binding of Gal-8 to MUC1, expressed by disseminating cancer cells, leads to their adhesion to the vascular endothelium, a crucial step in cancer metastasis (50). Gal-8 also binds to ALCAM/CD166, a member of the immunoglobulin superfamily that promotes homophilic and heterophilic cell–cell interactions (44). The ALCAM/Gal-8 complex modulates ALCAM–ALCAM intercellular contacts, thereby altering cell dissemination, and metastasis in neoplastic settings (44).

In contrast to the above, excess soluble Gal-8 interacts both with cell surface integrins and soluble ECM proteins to inhibit cell-matrix interactions. In that respect soluble Gal-8 resembles other soluble ECM proteins like laminin (68) and fibronectin (69, 70) that impair cell adhesion to immobilized integrin ligands. Soluble Gal-8 can induce the internalization of cell surface integrins and in such a way also impair cell adhesion. The effects of soluble Gal-8 resemble the effects of Gal-1 and Gal-3 that induce internalization of cell adhesion receptors (71), or can induce steric hindrance when bound to cellular receptors or to matrix proteins (72). In summary, the anti-adhesive effects of soluble Gal-8 could be mediated either upon its direct binding to integrins or other cell surface proteins, or alternatively, upon its binding and recruitment to the cell surface of ECM proteins such as fibronectin that, when soluble, exert anti-adhesive effects of their own (69, 70). This function requires the occupancy of both CRDs of the native Gal-8, and might account for the inability of the truncated monovalent soluble Gal-8N to inhibit cell adhesion (19).

The opposing effects of Gal-8 on cell adhesion are linked to the question why immobilized Gal-8 promotes cell adhesion and growth (33, 65), whereas soluble Gal-8 acts as a cytostatic factor (19). These opposing effects could be attributed, for example, to the different concentrations of the lectin, experienced by the cells. When Gal-8 is present at low concentrations, an immobilized ligand, it interacts only with high-affinity receptors of the integrin family (19) that promote cell migration and growth. In contrast, when Gal-8 is present at high enough concentrations as a soluble ligand, or when it is overexpressed, it can interact with low-affinity receptors that trigger its cytostatic effects. These receptors could either be other members of the integrin family or other cell surface proteins.
receptors altogether. Support to this model is provided by the fact that binding of Gal-8 to low- and high-affinity receptors results in a different repertoire of signals emitted by the cells. When cells adhere to immobile galectin-8 and only high affinity receptors are engaged, it triggers robust activation of the PI3K and MAPK pathways (33, 65). In contrast, when applied at high enough doses as a soluble ligand, Gal-8 triggers a delayed response that involves activation of stress activated kinases like JNK, expression of p21, inhibition of cell cycle progression and induction of cytostatic effects (35). This dual mode of action of Gal-8 may account for its ability to promote the progression of a number of cancer cells, yet inhibit growth of others [cf. (40, 52–54, 73)].

ii. Gal-8, epithelial-mesenchymal transition (EMT), and cancer metastasis

EMT is a reversible multistep process defined by the loss of epithelial characteristics and the acquisition of a mesenchymal phenotype. This transition is accompanied by disassembly of cell–cell contacts; loss of cell polarity; acquisition of spindle-shaped morphology; high cell motility; and invasiveness (74, 75). EMT is triggered by various growth factors and cytokines (75) that leads to activation of transcription factors such as Twist1 and Snail (76, 77). Despite the importance of EMT in propagating cancer metastasis (74, 75), the role of galectins in this process only begins to emerge (78–80). A clue as to the function of Gal-8 in this process, recently emerged when it was shown (81) that Gal-8 binds the Urokinase plasminogen activated receptor (uPAR) a key player in the induction of EMT (82). uPAR forms complexes (83, 84) with LRP1 (low-density lipoprotein receptor-related protein 1) (85) and MRC2 (mannose receptor C, type 2) (86) that also serve as binding partners to Gal-8, forming a [uPAR-LRP1-MRC2-Gal-8] complexes (81). Of interest, the extracellular Hsp90 (eHsp90) (87), which serves as a co-receptor of LRPI, has been implicated in the regulation of EMT in prostate cancer (88), thus linking LRPI to EMT as well. MRC2, the third member of the complex, is also part of the EMT. However, unlike uPAR and LRPI that positively regulate EMT, MRC2 is a negative regulator of this process, at least in prostate cancer. MRC2 acts by forming a complex with CD147 that is indispensable for the stability of three-dimensional acini formed by non-transformed prostate epithelial cells. By inhibiting the breakage of epithelial cell–cell contacts that maintain normal glandular tissue homeostasis, MRC2 prevents the onset of EMT (89). Finally, Gal-8 interactions with the cell adhesion molecule ALCAM (44) might also affect EMT, given that ALCAM is a negative regulator of this process (90). The above studies indicate that Gal-8 plays an intricate role in EMT, being a binding partner of both positive and negative modulators of EMT.

Gal-8 also promotes expression of the matrix metalloproteinase MMP9 (Shatz-Azoulay et al. unpublished), an important player in EMT (91). MMP9 has been shown to cleave the extracellular domain of E-cadherin (92). E-cadherin cleavage reduces the association of the intracellular domain of E-cadherin with β-catenin, and the cell’s actin cytoskeleton. This results in nuclear translocation of β-catenin (93) and subsequent activation of downstream effectors (e.g. Snail, Slug, Twist and ZEB1) that leads to increased expression of mesenchymal markers and decreased expression of epithelial markers (75). At the same time Gal-8 inhibits expression of bone morphogenetic factor-7 (BMP-7) (94), known as inhibitor of EMT (95), and a promoter of the reverse mesenchymal-epithelial transition (MET) (96). Decreased BMP-7 expression during EMT in human breast cancer contributes to the acquisition of a bone metastatic phenotype (95), implicating Gal-8 as a promoter of EMT and cancer metastasis. Still, metastasizing cancer cells must shed their mesenchymal phenotype via MET during the course of secondary tumor formation at the metastatic niche that resembles the primary tumor from which they arose (75). Hence, the role of Gal-8 in regulating both EMT and MET should be the subject of future investigation.

iii. Gal-8, inflammation, tumorigenesis, and metastasis

A close connection exists between inflammation and tumorigenesis (97, 98). Solid tumors are typically infiltrated with immune cells that can play an anti-tumorigenic role especially in blood; yet, inflammatory processes can play a pivotal pro-tumorigenic role at different stages of primary tumor development and formation of metastasis, including initiation, promotion, malignant conversion, invasion, neoangiogenesis, intravasation, dissemination, extravasation and adhesion of the tumor cells at the metastatic niche (99–101). For example, inflammatory mediators often activate oncogenic transcription factors, such as NFκB and STAT3, thus stimulating tumor invasiveness and metastatic dissemination (99). Similarly, chemokines that are peptide-signaling cytokines act as chemo-attractants that stimulate the migration of malignant cells towards their metastatic niche (99). Indeed, chemokine receptors are expressed by a variety of cancer cells (102) and up-regulation of [chemokines-receptor] pairs promote metastasis (99). This also benefits cancer cells by elevating their ability to express and secrete matrix metalloproteinases like MMP9 and MMP10 (103, 104). As an example, Monocyte chemoattractant protein-1 (MCP-1), also known as CCL2, and its receptor CCR2, are major players in promoting tumorigenesis and metastasis and high levels of MCP-1 were linked to cancer progression and poor prognosis in prostate, colon, breast, and cervical cancer (105). IL-6 promotes cancer progression and metastasis by stimulating cancer cell pro-
liferation, angiogenesis, cell adhesion and systemic suppression of host anti-tumor immunity (106). Another example is RANKL (receptor activator nuclear factor-κB ligand) and its receptor RANK (receptor activator nuclear factor), members of the TNF and TNFR superfamilies. RANKL, expressed by osteoblasts, promotes osteoclastogenesis and bone resorption through binding to RANK under physiological conditions and normal bone remodeling (107, 108). Yet, RANK is frequently expressed by cancer cells, whereas RANKL is frequently detected in the tumor microenvironment. Together they mediate cancer development (109), and are key players in the induction of osteolytic bone metastasis (110).

The role of galectins in controlling immune regulatory cancer networks has been explored (37) and a number of studies established the ability of Gal-8 to induce expression and secretion of cytokines and chemokines [e.g. (111)]. Gal-8-treated human microvascular endothelial cells (HMEC-1) produce CXCL1 (GRO-α), GM-CSF, IL-6, CCL5 (RANTES), CCL2 (MCP-1), CXCL3 (GRO-γ) and CXCL8 (IL-8), in a process that requires activation of NFκB (112). Gal-8 also increases secretion of a number of chemokines and cytokines (i.e., IL-3, IL-2, IL-6, TNF-α, MCP-1, and MCP-5) from bone marrow-derived dendritic cells (113). Most relevant are the observations that increased circulating Gal-8 in
the serum of cancer patients interacts with blood vascular endothelium and promotes secretion to the circulation of G-CSF, IL-6 and MCP-1. This results in increased expression of cell surface adhesion molecules on endothelial cells that triggers endothelial–cancer cell interactions (111).

We have recently shown that Gal-8 induces RANKL expression, osteoclastogenesis, and bone mass reduction in mice (94), pointing at Gal-8 as a potential modulator of cytokines expression in animal models and cancer cells in vivo. In support of this hypothesis, a strong correlation exists between expression of Gal-8 and IL-6 (Fig. 3) or MCP1 (CC12) (43) in some human solid tumors. These findings implicate cytokines and chemokines, whose expression is induced by Gal-8, as potential regulators of cancer homing to and propagation at the metastatic niche, which might be particularly relevant in the case of osteolytic bone metastasis (55, 114–117) (Fig. 4).

Fig. 5. The vicious cycle induced by Gal-8 to promote tumor growth and metastasis. Gal-8 drives cancer growth and metastasis by three mechanisms: i. Induction of cancer cell proliferation and promotion of EMT by autocrine and paracrine mechanisms that include induction of MMPs and inhibition of BMPs ii. Promotion of cell–matrix interactions that increase selective metastatic seeding through Gal-8 binding to $\alpha_3\beta_1$ and $\alpha_6\beta_1$ integrins, expressed by metastatic cells, that bind to Gal-8/fibronectin complexes at the metastatic niche; iii. Dissemination of Gal-8, expressed by the primary tumors, to target tissues, where it induces expression and secretion of a number of cytokines and chemokines that promote establishment of cancer cells at the metastatic niche. This generates a vicious cycle whereby the newly arrived cancer cells create metastatic lesions that further secrete Gal-8, thereby further enhancing the secretion of cytokines/chemokines by the metastatic microenvironment.
D. Conclusions

Collectively, the above studies offer a new, unexplored paradigm as to the role played by Gal-8 in the regulation of cancer growth and metastasis. They implicate Gal-8 as a driver of a ‘vicious cycle,’ whereby cancer cells that overexpress and secrete Gal-8, benefit from its potential to promote their own growth; potentiate EMT, and induce secretion of metastasis-promoting agents at the metastatic niche that induce further recruitment and seeding of the cancer cells. Studying the molecular mechanisms involved and identifying novel ways to breakdown this vicious cycle will inform development of Gal-8 inhibitors, either in the form of complex sugars or specific humanized monoclonal antibodies, as potential novel drugs for the treatment of cancer patients.

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References

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