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Glycobiology Insights

Role of Galectin-3 in Cancer Metastasis

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ABSTRACT: Galectins are a family of proteins that contain a canonical carbohydrate-recognition domain (CRD) with affinity for beta-galactosides. Within this family, an unique member, the chimeric, galectin-3, may be found in the cytoplasm and nucleus, and on the cell surface, besides being released into the extracellular space. Galectin-3 interactions with certain glycans and extracellular matrix (ECM) proteins have been described to promote and/or antagonize tumor cell apoptosis, to induce endothelial cell proliferation and angiogenesis, and to promote tumor cell adhesion and invasion, thus both potentially facilitating and hindering metastasis. Moreover, although galectin-3 is expressed in several types of malignancies and its expression has been correlated with transformation and metastasis-related events, its downregulation has also been associated with malignancy and tumor progression. These apparently conflicting data demonstrate that the role of galectin-3 in metastasis remains to be fully understood. Of course in nature, different cancer progression phenomena are simultaneously occurring in the many instances, where the patient has primary tumor and blood-borne and distant metastatic cells. This makes it all the more interesting to overview the role of galectins in cancer metastasis, especially galectin-3, since these and their related molecules are more than probable disease marker candidates and/or therapeutic targets.

KEYWORDS: galectin-3, galectin-3-ligands, cancer invasion, metastasis

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The Metastatic Process

Most tumors derive from clonal evolution of a single abnormal cell. Both genetic and epigenetic alterations are involved in this process. A single mutation does not give rise to a malignant tumor, and at least four steps can be implicated in malignant tumorigenesis: cell transformation, growth of transformed cells, local invasion, and distant metastasis.[1] Progression through these phases requires consecutive rounds of mutations and increased ability of tumor cells to flourish in their microenvironment. The tumor microenvironment is often a very harsh one, with low levels of oxygen and nutrients, and surrounding tissues that constitute a stressful barrier toward expansion. It is thought that this kind of microenvironment further propitiates adaptive mutations and increased aggressiveness.[2]

Distant metastases occurrence is the most common cause of cancer-related death. Metastasis is a complex process engaging many steps, regarding which much remains unknown.[3] During progression, tumor cells that are able to detach from primary tumor masses and travel to distant sites may succeed in founding new colonies through a complex series of coordinated events.[3,4] A large body of evidence presently suggests that tumor cells and their microenvironments profoundly influence each other. On the one hand, the tumor microenvironment has a major influence on the progression of the tumor

cells which, under a particular stimulus, such as hypoxia, tend to cross the tissues underlying the malignant growth in order to expand and invade new sites.[2] Moreover, the same *de novo* vessels formed by hypoxia-induced angiogenesis will support the primary growth and will also provide an escape route for invading cells, which will access the circulation through intravasation.[5] On the other hand, tumor cells produce several cytokines and growth factors reshaping the microenvironment that surrounds them.[6] Several biological aspects are implicated, and very initial metastasis steps unfold hand in hand with the development of the primary tumor itself. Crucial steps in the metastatic process include detachment of tumor cells from primary sites by loss of homotypic (between cancer cells) and heterotypic adhesions (between tumor cells and the extracellular matrix (ECM)). Within vessels, tumor cells and endothelial cells adhesion and survival of tumor emboli in the bloodstream require hetero- and homotypic aggregations, respectively.[7] Arrest in a new organ, extravasation into the surrounding tissue, maintenance of growth through new adhesive/de-adhesive interactions, and angiogenesis in the metastatic tumor are also vital steps that are dependent on an adequate microenvironment in order to be successfully completed.[3] A metastasis-favorable microenvironment is, both at primary and secondary sites, dependent on the active crosstalk between the tumor stroma that includes inflammatory and endothelial cells as well as fibroblasts and the ECM and tumor cells.[8]

Glycans as Modulators of Tumor-microenvironment Interactions

The glycocalyx, a glycan layer covering the external cell surface, mediates crucial interactions between cells and their microenvironment. Glycans are polysaccharides or oligosaccharides attached (glycosylation) to a protein or a lipid solely or in multiple attachments, in conjunction with other glycans. This forms a glycoconjugate that can be designated as a glycoprotein, or glycolipid.[9] Glycosylation is hence one of the most important post-translational modifications of proteins and lipids with regard to cell homeostasis. This phenomenon requires a large number of glycosyltransferases that constitute the specific enzymatic machinery involved in the biosynthesis and in the highly diverse structuring of glycans.[10] When compared to the normal glycosylation pattern of a certain cell type, tumor cells of the same tissue origin often express different carbohydrate conformations. Aberrant glycosylation is thus a common feature of malignancy. Length and branching of polylactosamine chains and the quantity and linkage type of terminal sialic acids are the most frequent cancer-associated glycan modifications.[9] Sialic acids, usually found in the terminal position or the non-reducing terminus of the carbohydrate,[11,12] affect the conformation of glycoproteins and allow recognition or masking of biological sites in molecules and cells.[13] Of the glycosylation modifications found in cancer, a group of tumor-associated antigens (TAA) with de novo or increased expression has long been recognized and includes the Thomsen–Friedenreich antigen (T antigen), its sialylated form, the Thomsen nouvelle (Tn) antigen, and Sialyl-Tn.[14] T antigen is an oncofetal glycan antigen and is the core-1 structure of O-linked mucin-type glycans (Galβ1-3GalNAcα1-Ser/Thr). The T antigen, often carried by MUC1, is generated by the T-synthase, which initiates the synthesis of core-1-derived O-glycans.[15] The core-1 disaccharide is a substrate for a number of sialyltransferases that synthesize different forms of the sialylated T antigen, including ST3Gal-I,[16] ST6GalNac-I, and ST6GalNac-II.[17] In the normal epithelium, this antigen is concealed by sialic acids, sulfates, or addition of other sugar chains to form branched and complex O-glycans. Unsialylated T antigen occurs in about 90% of all human cancers[18–20] and has been associated with disease progression.[21] In cancer, alterations of the glycosylation pattern are also frequently found in the end structures of the carbohydrate chains. Among others, TAA Sialyl-Lewis^X (SLe^X) and Sialyl-Lewis^A (SLe^A) are also frequently overexpressed in breast cancer and are associated with invasive capacity.[22–24] These glycosylation changes often reflect a deregulation of glycosyltransferase gene expression. Several studies have shown that glycosyltransferase genes, such as ST6Gal-I (N-acetyllacosaminide alpha-2,6-sialyltransferase)[25] and Mgat5 (alpha-1,6-mannosylglycoprotein 6-beta-N-acetylglucosaminyltransferase A),[26] are regulated by oncogenes. Several cellular models have been developed with the purpose of studying the mechanisms by which carbohydrate antigens might support cancer progression and aggressiveness, and showed that TAA are implicated in cell adhesion, migration, proliferation, and tumor growth.[27,28]

Glycan Receptors: Lectins as Regulators of Glycan-mediated Tumor-microenvironment Interactions

Reversible steps of homotypic and heterotypic cell–cell and cell–ECM adhesions are mediated by specific interactions between tumor cell-surface glycan-binding proteins, lectins, and their ligands present on other cells and/or within the tumor microenvironment.[29] Animal lectins are by definition proteins of non-immune origin that agglomerate or precipitate glycoconjugates. Intracellular lectins function in the trafficking, sorting, and targeting of maturing glycoproteins. Among them are the calnexin family (M-type, L-type, and P-type) lectins.[30] Extracellular lectins are either secreted into the ECM or body fluids, or localize at the plasma membrane, and mediate several functions including cell adhesion, cell signaling, glycoprotein clearance, and pathogen recognition. They include C-type lectins, R-type lectins, and siglecs. Galectins are the only family of animal lectins known to act both intra- and extracellularly.[31] They are considered master regulators of the information contained in the sugar code.

Galectins

Galectins are a family of lectins (carbohydrate-binding proteins) that share a common affinity for beta-galactosides and are

implicated in multiple cellular functions such as cell–cell and cell–ECM adhesions and apoptosis, among many others.[31,32] These lectins are abundant in epithelial and immune cells of animals,[33] and contain at least one carbohydrate-recognition domain (CRD).[34] To date, 15 members of the galectin family have been identified, cloned, and classified into three subgroups, based on their structure and number of CRD. These groups are (1) prototype galectins, which include galectin-1, -2, -5, -7, -10, -11, -13, -14, and -15; (2) chimera-type galectins, of which galectin-3 is the sole element; and (3) the tandem-repeat-type galectins, which include galectin-6, -8, -9, and -12.[32,35]

Most galectins are ubiquitously expressed in several human tissues. In malignant tumors, galectins may be found either silenced or upregulated when compared with the normal tissue. These proteins are believed to play key roles in several oncogenic processes.[36] Galectins, namely, galectin-1 and -3, mediate cell–cell and cell–ECM interactions. They are implicated in several key steps of the metastatic process[31] and cancer drug resistance.[37,38] However, the relationship between the presence of these lectins and tumor behavior is often found to be controversial, and the mechanisms by which they enhance metastasis remain unclear.[39,40] The present review attempts to uncover regulating mechanisms underlying the functions of galectin-3 in cancer and relate them to the lectin's role in tumor progression and metastasis.

Galectin-3

Galectin-3 has been one of the most well-studied members of the galectin family of soluble mammalian lectins.[41] It is encoded by a single gene, *LGALS3*, located on chromosome 14, locus q21–q22.[32] The gene is composed of six exons and five introns, spanning a total of ~17 kb (kilobase). There are two transcription initiation sites located 52 and 50 nucleotides upstream of exon I. The translation start site is in exon II. The ribonucleoprotein-like N-terminal domain, containing the proline–glycine–alanine–tyrosine (PGAY) repeat motif, is found entirely within the exon III. The carbohydrate-recognition sequence is found entirely within exon V.[42] Human galectin-3 is a 31-kDa chimeric protein. This unique galectin consists of three structural domains: an NH2 terminal domain containing a serine phosphorylation site, which is important in regulating its cellular signaling activity; a collagen-α-like sequence rich in glycine, tyrosine, and proline cleavable by matrix metalloproteinases; and a COOH terminal domain with a globular structure containing a single CRD, which recognizes beta-galactosides.[43–45] Galectin-3 is a monomer in solution; however, it forms pentamers via the flexible N-terminal domains upon binding to its saccharide ligands. Galectin-3 is secreted from the cell via a non-classic secretion pathway into the extracellular space.[46,47] Therefore, besides being present in the cell cytoplasm and nucleus, it can also be found at the cell surface and in the ECM.[48]

Galectin-3 is expressed in normal epithelial cells, activated T-cells, and fibroblasts.[49] Galectin-3 present at the cell surface and in the extracellular space binds to its numerous extracellular counterpart binding sites present in several ligands such as integrins, mucins, and growth factor receptors. By cross-linking glycosylated membrane receptors via binding their glycan parts, galectin-3 plays distinct cell-type specific functions.[50,51] These cross-links not only delay glycoprotein receptors' removal from the cell surface by constitutive endocytosis but also promote crosstalk between these proteins, thus modulating several signaling pathways.[52] Galectin-3 is therefore involved in carbohydrate-mediated processes such as cell adhesion, cell–cell interaction, cell migration, and cell signaling, and is a proapoptotic stimulus to T-cells[53,54] (Fig. 1). Cell-surface galectin-3 mediates homotypic cell adhesion.[55] Interestingly, the lectin was also found to be a determinant in the epithelial polarity program by functioning in the formation and/or stability of the centrosomes.[56]

Increases or decreases in the expression of galectin-3 have been associated with malignant progression of several cancers. The expression of galectin-3 is found to be upregulated in gastric, liver, and thyroid cancers, while it is downregulated in prostate,[57] head, and neck cancers, and uterine sarcoma when compared to normal tissues.[35,58,59] Decreased expression of galectin-3 is found and has been associated with a poorer prognosis in human breast cancer.[60] Despite the multiple contradictory findings in experimental studies and even in the few reported studies on human cancer specimen, galectin-3 is considered a promising potential therapeutic target in many different cancer types.[31,61]

In cancer, galectin-3 binds and interacts with a large number of glycoconjugates both intra- and extracellularly, regulating many biological functions and signaling pathways.[49,62] Among them are cell proliferation,[63–65] apoptosis resistance,[66–68] cell–ECM adhesion,[34,69–72] cell–cell adhesion,[41] cell differentiation,[73] and angiogenesis.[31,61] Specific subcellular localization of galectin-3 has been shown to be crucial to its functions in several models. Nucleoporin Nup98, for instance, mediates galectin-3 nuclear-cytoplasmic translocation, and thus, galectin-3 and β-catenin signaling pathways in regulating cell proliferation.[74] The most studied function of galectin-3 is its control of cell apoptosis. Remarkably, this is also the function that may be most interfered with by galectin-3's subcellular distribution. Cytoplasmic galectin-3 protects breast cancer cells from apoptosis by inducing cyclin D1, thereby promoting cell cycle arrest at an anoikis insensitive point (late G1),[75] improving cell adhesion properties,[76] inducing free radical-resistant cell survival,[77] protecting against inducible nitric oxide synthase (iNOS)-induced cytotoxicity,[78] impairing genistein-mediated apoptosis,[79] and binding and activating anti-apoptotic K-Ras.[80] Galectin-3 binds to CD45 on diffuse large B-cell lymphoma cells to regulate susceptibility to cell death.[81] Moreover, galectin-3 silencing inhibits epirubicin-induced ATP binding cassette transporters and activates the mitochondrial apoptosis pathway via β-catenin/GSK-3β modulation in colorectal carcinoma.[82] In addition, galectin-3 mediates the migration

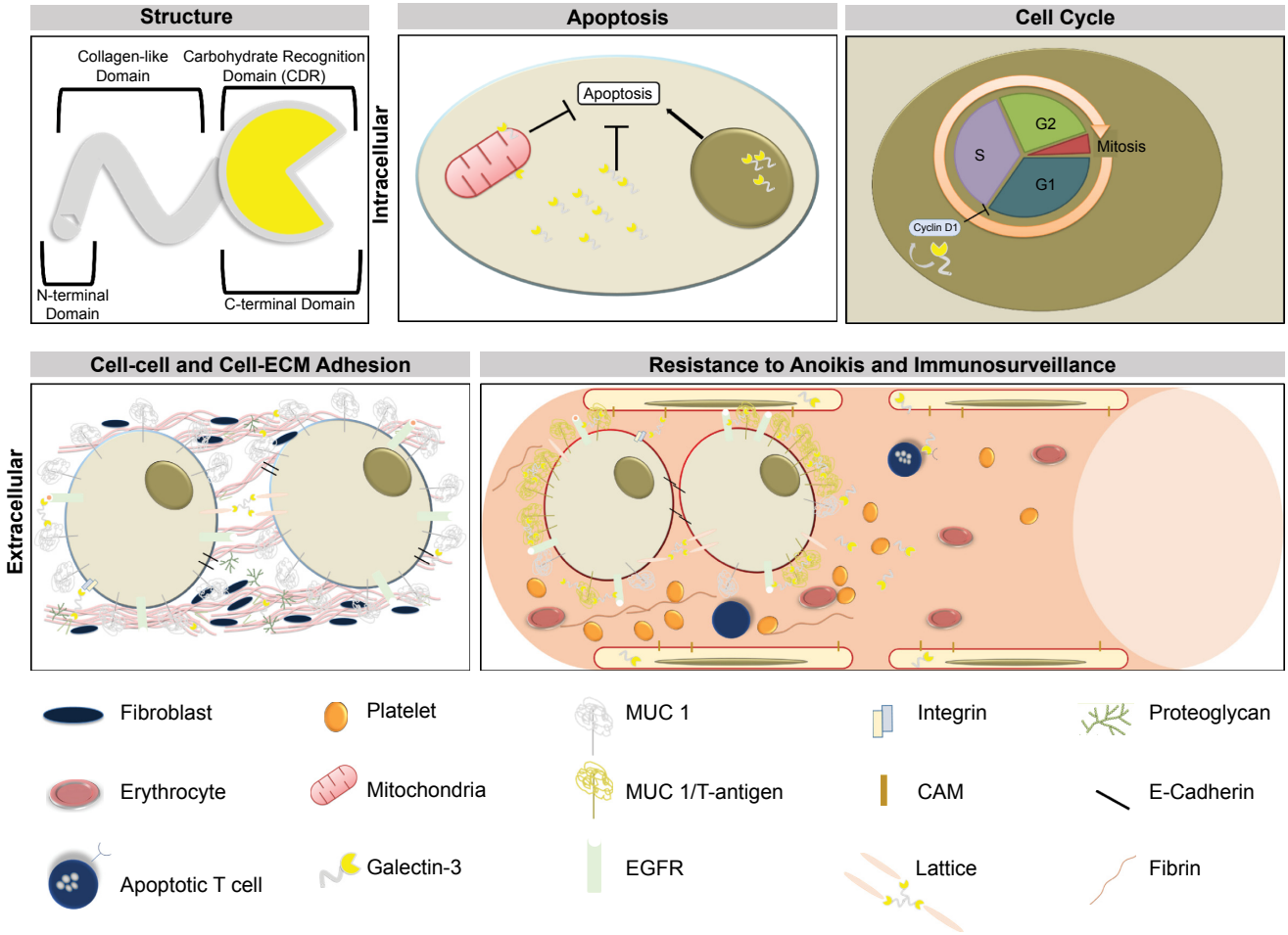

Figure 1. Structure, and intra- and extracellular functions of galectin-3. Galectin contains three structural domains: an NH2 terminal domain important in regulating its cellular signaling, a collagen-α-like sequence cleavable by matrix metalloproteinases, and a COOH terminal domain with a single carbohydrate-recognition domain (CRD). Intracellular galectin-3 both promotes and abrogates programmed cell death by apoptosis. This is dependent on its subcellular localization. Cytoplasmic galectin-3 protects cancer cells from apoptosis by promoting cell cycle arrest at late G1. Adhesive functions of galectin-3 are impaired by downregulation of the lectin, which along with MUC1 overexpression decreases cell–cell cohesion. Decreased galectin binding in the ECM facilitates tumor cell their movement throughout the ECM. Within vessels, galectin-3 high-expressing tumor cells, avoid anoikis by forming homotypic cell aggregates through binding to MUC1-carried T antigen. Galectin-3 also promotes adhesion between tumor and endothelium cells.

and invasion of tongue cancer cells *in vitro* via regulating the Wnt/β-catenin signaling pathway and Akt phosphorylation,[83] and also the progression of Oral tongue squamous cell carcinoma (OTSCC) via activation of the Wnt/β-catenin signaling pathway.[84] In contrast, expression of galectin-3 in breast cancer cells has also been found to inactivate Akt and sensitize them to TNF-related apoptosis-inducing ligand (TRAIL)-induced apoptosis[82] by upregulating PTEN.[33] De novo galectin-3 expression influences the response of melanoma cells to isatin-Schiff base copper(II) complex-induced oxidative stimulus. The presence of the lectin in the mitochondria favors increased Reactive oxygen species (ROS) production, thereby inducing oxidative cellular damage and apoptotic death.[85] These contradictory findings are likely to be related to galectin-3 cellular localization and whether or not it is phosphorylated. In particular, cytoplasmic galectin-3 seems to protect the cell from apoptosis, while nuclear galectin-3 has the opposite effect.[48] Phosphorylation at Ser6 is crucial for galectin-3 anti-apoptotic function;[67] it is also needed for the export of the lectin from the nucleus upon an apoptotic stimulus. Moreover, phosphorylated galectin-3 upregulates the MAPK pathway involved in regulating apoptosis.[86] In fact, galectin-3 has great sequence similarity to the apoptosis suppressor BCL2. Specifically, it contains an anti-death motif which, if mutated, abrogates its anti-apoptotic function.[87] Subcellular localization of galectin-3 also seems to be important for its cell growth effects. Galectin-3 expression in different cancer types, functions, and main mechanisms involved are summarized in Table 1.

Galectin-3 and Galectin-3-ligands in Primary Tumors: Coordination Toward Invasion

Galectin-3 was shown to be downregulated in primary malignant tumors when compared to benign mammary tumors,[88] suggesting a selective advantage for malignant growth in the presence of decreased levels of the lectin.[60,89] Recently, a study aiming to assess the clinicopathological significance of decreased

Table 1.

GALECTIN-3				
CANCER TYPE	**EXPRESSION**	**ACTION**	**MECHANISM**	**REFERENCE**
Prostate	Down-regulation	Apoptosis Metastasis	Cell adhesion to endothelium; homtypic aggregation; migration transendothelial; clonogenic growth; p21 stability regulation	35,57,58,68
Head and neck	Down-regulation	Differentiation; Metastasis	Loss association to dedifferentiation	73
Uterine	Down-regulation	Immunosuppression; Invasion	Expressed in fibroblasts, immune and cancer cells	54,59,71
Breast	Down-regulation	Adhesion; Apoptosis, invasion and metastasis	Adhesion of BT549 cell line to laninin, collagen IV; MMPs;	60,90,122
Thyroid	Up-regulation	Apotosis	Gal-3/Bax heterodimetrization, anti-apoptotic	66,91
Gastric	Down/up-regulation	Tumorigenesis; invasion	Decreased galectin-3 leads to a decrease of SKp2 and an increase of p27(KiP1); Galectin-3 up-regulates PAR1 and MMP1	65,70,72
Liver	Up-regulation	Cell proliferation; Migration and invasion	Anexin A7/galectin-3 complex affect cell cycle check point	64,177

galectin-3 expression and the long-term prognosis in patients with breast cancer showed that it was associated with tumor vascular invasion and metastases occurrence.[90] In accordance, reduced galectin-3 expression was associated with the presence of distant metastases in gastric cancer patients and with a higher invasive phenotype *in vitro*.[72] Downregulation of galectin-3 expression also resulted in increased apoptotic potential and decreased metastatic potential of prostate cancer cells in vitro.[68] There are nevertheless reports of high galectin-3 expression and increased invasiveness, particularly in thyroid carcinoma.[91]

It is consensual that the microenvironment influences the development of primary tumors.[2] Galectin-3 nuclear expression, thought to be promoting apoptosis in vitro,[48] was significantly lost, and expression of galectin-3 observed in malignant mammary tumors was mainly cytoplasmic when compared to benign tumors.[88] It thus seems that both the levels and subcellular localization of the lectin might be related with increased primary mammary tumor aggressiveness.[92,93] Corroborating a microenvironment-dependent regulation of galectin-3, despite homogeneous expression of the lectin by the mammary cancer cells *in vitro*, a dramatic decrease in its level of expression is present in primary tumor mice xenografts from the same cells.[88] Knowing that galectin-3 promotes homotypic aggregation between tumor cells *in vitro*[50] and that one of its putative ligands, MUC1, is responsible for de-adhesion between tumor cells in experimental settings,[94,95] one may wonder whether the mucin could, in addition to decreased galectin-3, be responsible for increased aggressive capacity of malignant tumors. As a matter of fact, MUC1 overexpression in malignant mammary tumors is observed to be significantly associated with distant metastases development.[96] When comparing the expression of the two proteins, a significant association between decreased expression of galectin-3 and increased MUC1 expression was observed in a malignant mammary tumor series.[97] A hint on a possible regulatory loop between the two is present in the work of Ramasamy in breast cancer cell lines. This work showed that by inhibiting MUC1 in BT549 malignant cell lines, a decrease in galectin-3 expression is observed.[98] Data also demonstrated an increased MUC1 expression, upon galectin-3 inhibition in mammary tumors.[97] Moreover, downregulation of MUC1 was also observed in BT549 breast cancer cell line upon transfection with galectin-3.[99] Altogether, these data point to the existence of a feedback regulatory loop between galectin-3 and MUC1 in mammary gland tumors. An N-glycan has been suggested to be involved in the ability of MUC1 to regulate galectin-3 expression.[98] Despite overexpression of MUC1 in mammary tumors, there is a low expression of galectin-3-binding sites *in vivo*.[97,100] Sialylation may impair galectin-3 binding to its putative ligands in a few non-mammary tumor contexts.[101,102] A neuraminidase cleavage of sialic acids in malignant mammary tumors also restores the ability of the lectin to bind to the tissues. Furthermore, the presence of α2,6-linkage is shown in areas with decreased galectin-3-binding sites expression, thus further suggesting that α2,6-linked sialic acid is at least in part responsible for switching off galectin-3 binding in mammary tumors *in vivo*.[97] Galectin-3-binding-sites expression was consistently observed at the periphery of tumor xenografts and in spontaneous malignant mammary tumors pointing to a microenvironment-dependent regulation of the type of glycosylation during mammary tumor progression.[97]

Galectin-3 mediates cell–ECM heterotypic adhesion processes, which may modulate tumor cells' detachment from the primary site and, therefore, invasion.[103] However, contradictory results in the literature show that modulation of galectin-3 functions can both increase and decrease adhesion of cells to its ECM protein ligands, laminin, collagen type IV, fibronectin, and vitronectin. Using different approaches, divergent effects were observed on tumor growth.[76,104–107]

Extracellular galectin-3 was found to increase adhesion to elastin and enhance cellular proliferation.[107] Remarkably, breast cancer cells were found not to constitutively secrete galectin-3 but rather to respond to stress by triggering a mechanosensitive mechanism and other external stimuli inducing the rapid externalization of the lectin with subsequent faster adhesion and spreading.[108] Conversely, extracellular galectin-3 negatively regulates attachment and spreading of retinal pigment epithelial cells through its ability to cross-link glycans.[109] Restriction of growth and expansion of the epithelium has been proposed to occur because of stabilization and/or modulation of basal interactions between cells and the ECM by galectin-3, and can be reversed by anti-galectin antibodies.[110] An elegant work by Kariya showed that cell migration through the ECM is dependent both on a low concentration of the lectin and glycosylation of its ECM putative ligands such as laminin. Notably, a high concentration of galectin-3 completely inhibited cell adhesion to the ECM because of induced homotypic aggregation.[111] However, galectin-3 silenced MDCK[112] cells adhered less to laminin-111, collagen type I, and matrigel, and presented reduced proliferation.[113] Given that integrin antibodies inhibited the adhesion of MDCK cells to all substrates, this points to an indirect role of galectin-3 in cell–ECM adhesion probably by interacting with integrins modifying their avidity for their ligands.[114] As an illustration of the outside-in signaling, which regulates integrin activation and cell adhesion, the turnover of focal adhesions is interdependent both on extracellular galectin-3 and intracellular pY14Cav1, where galectin-3 binding was proposed to promote integrin clustering and formation of focal contacts in mammary cells.[115] Moreover, galectin-3- and phospho-caveolin-1-dependent outside-in integrin signaling mediates the EGF motogenic response in mammary cancer cells. In response to EGF, galectin-3 enables outside-in integrin signaling stimulating phospho-caveolin-1-dependent RhoA activation, actin reorganization in circular dorsal ruffles (CDRs), cell migration, and fibronectin remodeling.[116] In addition, silencing of RhoA significantly reduced the tumor growth; decreased the levels of galectin-3, β-catenin, MMP-9, and cyclin D1/2; and increased the levels of $p21^{CIP1/WAF1}$ and $p27^{Kip1}$. This led to inhibition of cell migration, invasion, and proliferation in a human tongue cancer *in vitro* model.[117] Galectin-3 also regulates p21 stability in human prostate cancer cells[68] and ablation of galectin-3-induced p27KIP1-dependent premature senescence without oncogenic stress.[118] In another model, immortalized corneal epithelial cells, galectin-3 activated the focal adhesion kinase (FAK), a key regulator of integrin-dependent cell signaling, and a member of Rho GTPases, Rac1 GTPase, which is known to play an important role in reorganizing the actin skeleton and the formation of lamellipodial extensions. The role of galectin-3 in promoting lamellipodia formation in this model was dependent on the N-glycosylation of the α3β1-integrin.[52] In the same model, a galectin-3-induced regulatory mechanism for increasing MMP9 metalloproteinase expression was responsible for disruption of cell–cell contacts required for cell motility in migrating epithelia.[119] In the process of cornea regeneration, galectin-3 is coordinately upregulated with glycosyltransferases responsible for the assembly of its glycan ligands, GnTIVb, β3GalT5, T-synthase, and ST3Gal-I, whereas glycogenes inhibiting glycan recognition by galectin-3 such as GnT-III, ST6galI, and ST8SialIV are downregulated.[120] Interestingly, specific glycan alterations such as α2,6 sialylation on β1-integrins have been shown to decrease galectin-3 binding, which is restored upon neuraminidase treatment in SW48 colonocyte cell line.[102] More recently, a functional feedback-loop between β1-integrins and galectin-3 that involves the epigenetic induction of galectin-3 expression during integrin-induced EMT and cell scattering was reported.[121] Notably, MMP9 was found to be aberrantly sialylated in breast cancer cells, thus presenting reduced binding to galectin-3. This aberrant glycosylation of MMP9 was suggested to bypass the holdback of its activity by the lectin, which along with the downregulation of galectin-3 might lead to more widespread matrix degradation, thus facilitating tumor cell invasion and angiogenic growth.[122] However, galectin-3 is itself a substrate for MMP-2 and MMP-9, and breast cancer cells harboring non-cleavable galectin-3 presented reduced tumor growth and angiogenesis.[123] More recently, extracellular galectin-3 was shown to accumulate because of the decrease in MMP-2 activity. Galectin-3 signaling events were also blocked because of an impaired interaction with 4F2hc, inducing an increased degradation of β-catenin.[124] Finally, galectin-3 was proposed to facilitate cell migration and invasion of melanoma cells *in vitro*, and to induce metastasis *in vivo*, in part through regulating the transcription activity of AP-1 and thereby upregulating MMP-1 expression.[125] In addition to the molecular expression profiles in malignant tumor cells that seem to favor tumor cell detachment from primary tumors, the tumor stroma is also most likely to be a crucial player in this process. A significantly decreased expression of galectin-3-binding sites was demonstrated in the ECM of malignant mammary tumors when compared to normal-adjacent tissue. The decrease in galectin-3-binding sites seems to be associated with increased expression of galectin-1 in the stroma of malignant mammary tumors.[88] Galectin-1, the other extensively studied galectin to date,[126] is a prototype galectin.[127,128] First, galectin-1 is able to bind components of the ECM such as laminin, fibronectin, and integrin,[46] as well as membrane glycoproteins and glycolipids present in adjacent cells, thereby modulating cell–ECM and cell–cell adhesions.[129] Second, galectin-1 is involved in cell proliferation,[130] apoptosis,[131] and even mRNA splicing.[132] Third, galectin-1 is able to induce apoptosis of activated T-cell and T-leukemia cells.[131] Galectin-1 expression, particularly in the tumor stroma, has been consistently associated with a poor differentiation and progression in several types of cancer.[133–137] As galectin-3, galectin-1 is considered a potential therapeutic

cancer target.[138] Galectin-1 functions are believed to have a major impact on the development of the malignant tumor. Galectin-1 participates in a variety of oncogenic processes, including transformation,[139] proliferation and cell cycle regulation,[140] cell adhesion and invasion,[141] metastasis,[142] and apoptosis in activated T-cells, which constitutes an important mechanism of tumor-immune escape.[131] Moreover, galectin-1 facilitates tumor progression since it is essential for tumor angiogenesis.[142] Galectin-1-expressing carcinoma cells can synthesize and secrete galectin-1 into the stroma using its non-classical secretory pathway,[143] or galectin-1 can be synthesized by stromal cells, especially stromal fibroblasts, as they get stimulated by oncologic signals from carcinoma cells or from ECM during ECM remodeling. Galectin-1 is known to promote lower strength cell–ECM adhesion when compared to galectin-3.[144] This seems to point to a role of impaired galectin-3-mediated cell–ECM adhesion in the acquisition of invasive capacity in mammary tumors. Ellerhorst et al showed increased expression of galectin-1 in the stroma of primary prostate carcinoma samples in comparison to the stroma of normal prostate tissues; moreover, increased galectin-1 expression positively correlated with poor prognosis.[145] Furthermore, a correlation between increases in expression of galectin-1 in cancer-associated stromal cells, and tumor invasiveness and tumor progression in breast cancer was shown by Jung et al.[137] In fact, a study aiming to determine upregulated proteins, in the fluid bathing the tumor cell microenvironment, as potential serological markers for early detection of breast cancer, identified galectin-1 as one of the 26 breast cancer potential markers.[146] Galectin-1 was also identified as a metastasis-associated protein by several studies, which demonstrated its upregulation in human breast carcinoma.[147] Unlike galectin-3, galectin-1 was found to promote intracellular accumulation of β1-integrin with concomitant decrease in its cell-surface expression.[148] Galectin-1 was found to be a better inhibitor of tumor cell adhesion to ECM components than galectin-3.[144] Finally, in SK-N-MC human neuroblastoma cell line, cleaved galectin-3, which has impaired ability to self-aggregate upon glycan interaction because of loss of its N-terminal domain, presented weaker binding properties and decreased capacity of competing with galectin-1 for the substrate.[149] Although these galectins present similar glycan affinities, there are slight differences, such as the more extensive CRD of galectin-1 for a complex glycan than for simple saccharides, which may account for important implications of galectin–glycan interactions at the cell surface.[150] The degree of branching in N-glycans and clustering of core-1 O-glycans are the positive modulators of galectin-3 avidity to its ligands.[151] Gabius' group found a correlation between decreased galectin-3 expression and increased binding potential for galectin-1 in lymph node metastases of breast cancer.[144]

In addition to increased galectin-1, an overall decrease in galactosylation of malignant tumor areas has also observed in mammary tumors. These alterations in the glycosylation pattern of the ECM may add to the impairment of adhesive functions of galectin-3 in malignant mammary tumors.[88,152] The expression of the GLT25D1 galactosyltransferase, involved in collagen glycosylation,[153] was dramatically decreased in malignant tumors when compared to normal mammary tissue. Knock-down of galectin-3 further downregulated the levels of GLT25D1 expression in CMT-U27 malignant mammary tumor cell line.[88]

Finally, galectin-3 is chemoattractant to endothelial cells and stimulates neovascularization in vivo, therefore contributing to tumor angiogenesis, an essential step for metastatic spreading.[154] Among other functions, galectin-3 accelerates M2 macrophage infiltration and angiogenesis in tumors.[155] In accordance, galectin-3 disruption impaired tumoral angiogenesis by reducing Vascular endothelial growth factor (VEGF) secretion from TGFβ1-induced macrophages.[156] In turn, VEGF-C enhanced cervical cancer invasiveness via upregulation of galectin-3 through the NF-κB pathway.[157] Elevated expression of galectin-3 in Lewis lung cancer tumor cells seems to contribute to the migration of myeloid-derived suppressor cells (MDSCs) to the tumor microenvironment in response to cisplatin.[158]

Galectin-3 and Galectin-3-ligands in Blood-borne Tumor Cells: Anoikis Survival

Interactions between tumor cells and the endothelium are important rate-limiting steps in metastasis.[7] Contrary to that observed in most areas of primary mammary tumors, intravascular malignant mammary tumor cells present increased expression of both galectin-3 and its ligands, MUC1 and EGFR, often also found in circulating tumor cells.[159] MUC1 and EGFR are observed focally at the blood-borne cells' membrane, a pattern that is not observed in sedentary cells of primary tumors. They coexpress with galectin-3 suggesting that the lectin could be clustering its ligands *in vivo* as hinted by experimental observations.[21,97] This may contribute to pro-survival signaling and homotypic aggregation-related anoikis resistance.[160] Galectin-3 physical interaction with MUC1 in tumor emboli in breast cancer cells was demonstrated to be dependent on the unsubstituted form of the T antigen in vitro.[160] This is a common glycoform of cancer-associated MUC1,[161] highly expressed in circulating breast cancer cells.[162] As opposed to the majority of malignant mammary tumor cells, intravascular tumor cells present increased galectin-3-binding sites expression, suggesting that at least some of its ligands would likely not be sialylated in this subpopulation either by downregulation of sialyltransferases[16,17] or by increased sialic acid cleavage.[163,164] The unsubstituted T antigen was found to be coexpressed with galectin-3 in tumor cells inside vessels. Supporting the hypothesis that intravascular tumor cells take advantage of galectin-3–MUC1 interactions in order to invade vessels and survive in the circulation avoiding anoikis, a proximity ligation assay[165] between the lectin and the MUC1-carried T antigen provided proof

of the occurrence of such interactions in tumor emboli of spontaneous malignant mammary tumors *in vivo*.[97] Furthermore, considering that MUC1-EGFR signaling is a pro-survival one and that galectin-3 is involved in bridging this interaction in breast cancer cells *in vitro*,[98] it further suggests that these are important mediators of circulating tumor cell survival in malignant mammary tumors. Moreover, regarding homotypic adhesion between tumor cells, galectin-3-induced clustering of MUC1 enables E-cadherin-mediated aggregation, hence promoting survival to anoikis in HBL-100 breast cancer cell line.[160] Inhibition of cellular aggregation occurs when lactose-functionalized G2-dendrimers provide competitive binding sites to galectin-3 putative cancer cell ligand, T-antigen on MUC1.[166] Adding to a direct interaction between MUC1 and EGFR,[95] MUC1–EGFR bridging by galectin-3 has also been demonstrated *in vitro* using BT549 breast cancer cell lines.[98] In a pancreatic cancer model, the opposite seems to occur: galectin-3 inhibition led to decreased levels of both MUC1 and EGFR, and the lectin seems to decrease MUC1–EGFR interactions as well as their expression at the cell surface.[167] Recruitment of EGFR by galectin-3 restricts its diffusion, limiting the receptor interaction with negative Cav1 microdomains and thereby promoting EGFR signaling and tumor growth, depending on GnT-V glycosylation.[168] Conversely, GnT-III-catalyzed N-glycans inhibit galectin-3 cross-linking of EGFR.[111] Notably, galectin-3 was found to be essential for EGF-mediated interactions between MUC1 and EGFR through an N-glycan present in the lectin's C-terminal subunit.[98] The fact that there is an increase in galectin-3 at the mRNA and protein levels in tumor emboli additionally suggests a crucial role of a specific microenvironment in promoting cell survival ability against stress-inducing conditions and thus invasion.[154,169] The increase in anoikis galectin-3-mediated resistance in this intravascular tumor cell subpopulation is further supported by the concomitantly demonstrated decrease in pro-anoikis galectin-1 expression in intravascular tumor cells.[170] An upregulation of galectin-3 with concomitant downregulation of galectin-1 is observed in inflammatory cells in vessels, and a similar mechanism could be happening during the metastatic cascade of malignant tumors.[171] Galectin-3 competes with galectin-1 for ligands at the cell surface, and is downregulated by p16INK4a.[172] Despite its previously described relationship with aggressiveness, galectin-1 is a pro-anoikis effector under the control of the tumor suppressor p16INK4a in pancreatic cancer.[170] In salivary gland tumors, when present, staining for the p16INK4a coincides with galectin-1 expression.[173] An interesting coordinated expression between the lectins and their N- and O-glycosylated ligands was also observed to be under the control of p16INK4a and to tune this anoikis effector system.[172] Moreover, unlike high levels of galectin-1, which prolong H-Ras and K-Ras activation of ERK while PI3-K activation is diminished, galectin-3 inhibits N-Ras and H-Ras activation,[174] being crucial, however, for EGF-stimulated increase in K-Ras-GTP, promoting specifically strong K-Ras activation of PI3-K and Raf-1 while attenuating ERK activation. This suggests that levels of galectin-1 and -3 varying among different subpopulations of tumor cells may define the outputs of oncogenic K-Ras.[175] A novel calcium-sensitive and PKC-dependent pathway through which circulating galectin-3 may promote cell migration while activating the ERK1/2 was described.[176] Furthermore, downregulation of galectin-3 leads to a decrease in uPAR levels via the MEK/ERK pathway and inhibits the proliferation, migration, and invasion of hepatocellular carcinoma cells.[177] In line with this, overexpressed galectin-3 in pancreatic cancer induces cell proliferation and invasion by binding Ras and activating Ras signaling.[178] Galectin-3 also plays an important role in regulating colon cancer cell migration and potential distal localization. The galectin-3 enhancement of cell migration is mediated through the K-Ras–Raf–Erk1/2 pathway.[179] Annexin A7 interaction with galectin-3 regulates tumor cell proliferation, attachment, migration and invasion of mouse hepatocellular carcinoma Hca-F/P cell lines, influencing lymphatic metastasis of tumors.[64] Furthermore, galectin-3 plays an important role in escape from immune surveillance. Extracellular galectin-3 requires specific cell-surface glycoprotein receptors to trigger T-cell death.[180] Induction of T-cell apoptosis by secreted galectin-3 is dependent on the presence or absence of cytoplasmic galectin-3.[181] Galectin-3 is a regulator of cell growth and apoptosis through a cell death inhibition pathway involving Bcl-2.[182] Adding to that, intracellular galecin-3 functions as an inhibitory regulator of T-cell activation by downregulating signal transduction and inhibiting cytokine production.[183] In a mouse tumor model, delivery of high doses of galectin-3 inhibited tumor-reactive T-cells and promoted tumor growth.[184]

Galectins and Galectin-ligands at Distant Sites: Adapting to a New Microenvironment

In tumor-endothelial cell adhesion assays *in vitro*, among other possibly contributing mechanisms, galectin-3 induces MUC1 clustering on the tumor cell surface by binding the T antigen. This exposes adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1), which facilitates the interaction between tumor and endothelial cells—heterotypic adhesion.[160] Galectin-3 thus mediates both homotypic aggregation of cells invading vessels and heterotypic tumor cell adhesion to endothelial cells, thereby preserving them in the blood stream and enabling their arrival at distant sites.[50] In this regard, the work of Glinsky's group showing that the microvascular endothelium of metastasis-prone tissues undergoes activation as a response to unsialylated T antigen present in circulating tumor cells and that this activation results in an increased expression of galectin-3 is very interesting. Interaction with the glycan causes a gradual decrease in tumor cell velocity, leading to arrest of breast cancer cells and retention in the microvasculature.[185] The same

group was also able to show that anti-T antigen and anti-galectin-3 antibodies, as well as galectin-3 inhibitors MCP and lactosyl-L-leucine inhibited >90% of breast cancer cell metastases occurring in lungs and bones of mice *in vivo*, further pointing to a crucial role of β-galactoside-mediated tumor–endothelial cell adhesion.[186] This does not contradict James Ewing's mechanical entrapment model,[3] which also seems to play a role, albeit may be a supportive one, in mediating metastatic cell arrest in the microvasculature of target organs.[186] Following the initial metastatic cell attachment to endothelial cells mediated by T-antigen/galectin-3 interactions, endothelial integrin α3β1 stabilizes tumor/endothelial cell adhesion and induces the formation of macromolecular signaling complex activating several major signaling pathways in endothelial cells.[187] Moreover, G3-C12 appears to inhibit T-antigen/galectin-3 and galectin-3/galectin-3 interactions *in vitro* and *in vivo* and to moderate early steps of the metastatic cascade leading to reduced carcinogenesis *in vivo*. Furthermore, it significantly reduced metastatic cell deposition and consequent outgrowth within the vasculature of mice.[188] In a melanoma model, galectin-3 was also found to promote adhesion of tumor cells and mediate lung colonization although through poly N-acetyllactosamine (polyLacNAc) N-glycan interactions and not the T antigen.[189] Cell-surface LAMP1 facilitates lung metastasis by providing ligands for galectin-3 that has been shown to be expressed in highest amounts on lungs and constitutively on its vascular endothelium.[190] PolyLacNAc on melanoma cells and galectin-3 on the lungs play critical roles in arrest and extravasation of cells in the lungs, and were proposed as targets to inhibit lung metastasis.[191] Following the initial clonogenic survival upon arrest at distant sites, vasculature and early ECM adhesion, in which galectin-3 has been implicated because of its anti-apoptotic[67,75] and homotypic adhesion functions,[46,50] in order to grow and locally invade, metastatic cells need to detach from each other.[3] In order for this to occur, a sustained high galectin-3 expression, as observed in intravascular tumor cells, would be damaging because of increased cell–cell adhesion and cell–ECM adhesion.[104,107] In accordance, metastatic lesions present a dramatic decrease in galectin-3 expression. Moreover, galectin-1 is strongly expressed in metastases as observed in primary tumors.[169]

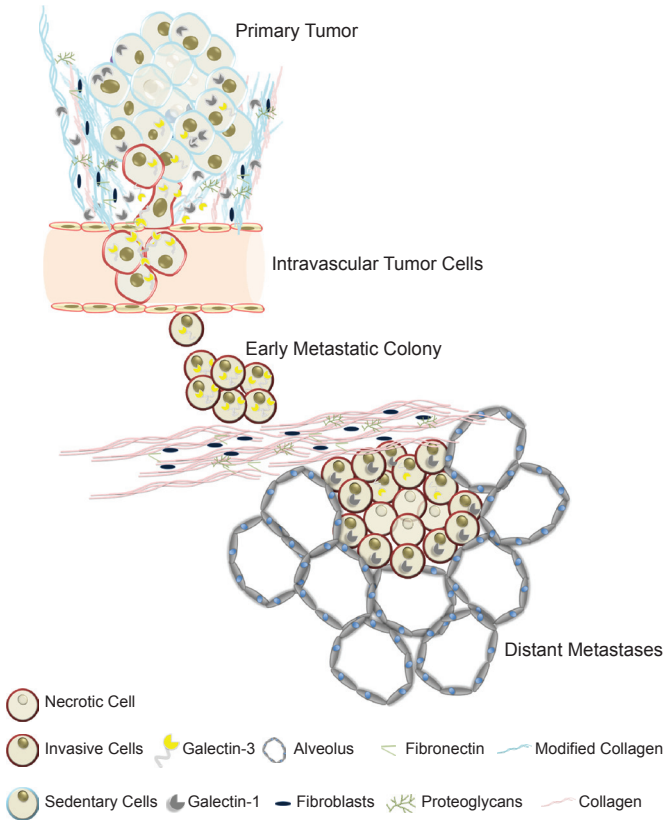

Figure 2. Galectin-3 in the metastatic process. Proposed model summarizing the role of galectin-3 in tumor progression and metastasis, correlating loss and/or gain of expression of galectins and their binding sites, both in tumor cells and within the tumor microenvironment. In malignant cells, downregulation of galectin-3 leads to decreased cell-cell cohesion. Additionally, decreased galectin-3-binding sites in the ECM and increased galectin-1 lead to a decreased adhesiveness of galectin-3-expressing tumor cells and facilitate their movement throughout the ECM. Within vessels, galectin-3 high/galectin-1 low-expressing tumor cells are able to avoid anoikis and to form homotypic cell aggregates further facilitated by decreased sialylation of galectin-3-ligands at the cell surface. Galectin-3 also facilitates tumor cell extravasation. Finally, in a target organ, low galectin-1-expressing, galectin-3-positive tumor cells are able to adhere to the normally glycosylated ECM (which possesses plenty of galectin-3-binding sites), thus forming an early metastatic colony. By then, the majority of the tumor cells will gradually downregulate or lose expression of galectin-3 and upregulate galectin-1 in order to proceed further in the growth establishment giving rise to well-established metastases.

Main Conclusions on the Role of Galectin-3 in Tumor Progression and Metastasis

Our better understanding of how malignancies progress into well-established distant metastases might enable the design of protocols to either prevent their appearance or achieve their regression. In the work presented here, we sought to better understand the mechanisms underlying the role of galectin-3 in tumor metastasis that could aid in future therapeutic-focused studies.[192] A model correlating the loss or gain of expression of galectins and their binding sites within the tumor microenvironment as well as its consequences for tumor metastasis may be proposed (Fig. 2):

- In malignant tumors, adhesive functions of galectin-3 are impaired by downregulation of the lectin. In addition, it may lead to MUC1 overexpression, further decreasing cell cohesion.[95]
- Differential glycosylation of the mucin, in specific tumor-microenvironment scenarios, might play a role in the mutual regulation of these proteins in malignant tumors.
- Decreased galectin-3-binding sites in the ECM additionally lead to a decreased adhesiveness of galectin-3-expressing tumor cells and facilitate their movement throughout the ECM. Increased galectin-1

not only occupies galectin-3-binding sites but also by itself facilitates tumor cell progression throughout the ECM.[144]

- Within vessels, galectin-3 high-/galectin-1 low-expressing tumor cells are able to avoid anoikis[172] and to form homotypic cell aggregates, further facilitated by decreased sialylation of galectin-3-ligands at the cell surface,[21,160] supporting not only cell survival but also metastatic cell arrest in microcirculation.[160] Also, by promoting interaction between tumor and endothelium cells, galectin-3 facilitates tumor cell extravasation through vascular endothelium.
- Lastly, once in a target organ, low galectin-1-expressing[193] and galectin-3-positive tumor cells are able to adhere to the normally glycosylated ECM (which possesses plenty of galectin-3-binding sites), thus forming a metastatic or a secondary tumor.[29] By then, the majority of the tumor cells will gradually downregulate or lose expression of galectin-3 and upregulate galectin-1 in order to proceed further in the growth establishment.[194–197]

Author Contributions

Analyzed the data: JdO, CR. Wrote the first draft of the manuscript: JdO. Contributed to the writing of the manuscript: JdO, CR. Agree with manuscript results and conclusions: FG. Jointly developed the structure and arguments for the paper: JdO, CR, FG. All authors reviewed and approved of the final manuscript.

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