Drugs of the Future 2008, 33(12): 1057-1069

CCC: 0377-8282/2008
DOI: 10.1358/dof.2008.033.12.1247541

Galectin-1 as potential therapeutic target for cancer progression

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Abstract

Galectins are a 15-member family of β-lactoside-specific lectins. They are expressed differentially in normal versus neoplastic tissues and are known to play important roles in biological processes pertinent to cell proliferation, death and migration. Galectin-1 is a hypoxia-regulated protein that exerts potent proangiogenic effects. It is expressed in a large set of human tumor types and is directly implicated in glioma cell migration. Galectin-1 also participates in the resistance of cancer cells to chemotherapy and radiotherapy, and is involved in the activation of the Ras oncogenic pathway. Lastly, galectin-1 is a major player in the tumor immune escape process. Targeting galectin-1 by means of specific antibodies or selective small molecules can not only impair tumor immune escape, cancer cell migration and tumor neoangiogenesis, but also restore a certain level of sensitivity in metastatic tumors to chemo- and radiotherapy. Indeed, antisense oligodeoxynucleotides (ODNs), small interfering RNAs (siRNAs) or selective small molecules can impair the protumoral intracellular functions of galectin-1. Thus, it represents a novel target to combat various types of cancers. This review will provide valuable information for clinicians and researchers who wish to gain an appreciation of the latest endeavors in this field and identify possible new lines of investigation in cancer.

The challenge of tumor metastasis

Metastases, as evident in the large majority of human solid tumors and locoregional invasion into surrounding tissues, are the biological processes that really kill cancer patients. In 2005, 1 in 4 patients in the Western world still died from their cancer, and of these, 90% died from metastases. Over nearly half a century of research on cancer therapeutics, the focus has been almost entirely on identifying drugs that result in the remission of primary cancers such as testicular, breast, prostate, cervical and small cell lung (SCLC) cancers. And yet, proportionately few people die as a result of the primary tumor (1). Rather it is when the cancer spreads that there is significant mortality. The treatment and prevention of metastases is a major unmet medical need, especially in the context of gliomas, melanomas, pancreatic cancers and non-small cell lung (NSCLC) cancers.

Metastatic cancers tend to affect vital organs where the high doses of anticancer chemotherapeutic agents that would be needed to challenge the emerging tumors would cause substantial collateral damage (2). Treatment options that block the process of metastasis would be expected to have a significant effect on cancer mortality, particularly where they are used alongside treatments challenging the primary tumor. This has been the drive of recent research into potential new treatments (1).

In metastasis, cancer cells from a primary tumor start migrating and seeding around the body. Metastatic tumors often express the cell type of the primary tumor, so that liver tumors resulting from the metastasis of breast cancer will consist of proliferating undifferentiated breast cells (3). The process of metastasis begins with cells in the primary tumor climbing over or around neighboring cells (3, 4). They do so by adjusting their cytoskeletons in order to adhere to other cells and to the extracellular matrix (ECM) via proteins expressed on the outside of their plasma membranes. By extending the cell to adhere to an adjacent cell and releasing adhesion at

the back, cells can migrate forward (3, 5, 6). Once the cells reach the basal lamina surrounding the tumor tissue, they secrete matrix metalloproteinases (MMPs) that act as “molecular scissors” to cut through the thick layer of proteins and thus allow the cell to pass through and make contact with blood or lymph cells (7, 8). By the same process of cell adhesion, the cancer cells can then anchor themselves to mobile blood cells or lymphocytes, which allow them to be transported around the body to be deposited in vital organs, where they proliferate to form metastatic tumors (3, 9). These migrating cancer cells are unfortunately resistant to apoptosis (6).

A further unfortunate fact is that approximately 80% of the drugs used today to fight cancer are proapoptotic (10, 11). The resistance of migrating cancer cells to apoptosis explains, at least partly, why highly invasive and/or metastatic cancers are largely resistant to modern chemotherapy and radiotherapy (6, 11). However, more recent published data indicate that compounds able to slow down the migration of apoptosis-resistant cancer cells are also able to restore a certain level of apoptosis sensitivity in these resistant migratory cells, especially in glioblastomas, the ultimate level of malignancy in the glioma group of tumors (12-16). Therefore, a compound that is able to reduce the migration, locoregional invasion and metastatic potential of cancer cells will not only delay these processes, but also restore a certain level of radio- and chemosensitivity to conventional cytotoxic drugs, which should promote cancer cell death.

In addition to the above-mentioned biological phenomena, the poor prognosis associated with malignant tumors and metastasis is often related to tumor immune escape, i.e., the result of a number of mechanisms that contribute to tumor cell evasion from the body’s own natural immune response (17, 18).

The concept of galectin-1 as a potential therapeutic anticancer target

What is galectin-1?

Galectins are a 15-member family of carbohydrate-binding proteins with high affinity for β-galactosides, found in mammals. They all share a consensus of amino acid sequences of about 130 amino acids and a carbohydrate recognition domain (CRD) responsible for β-galactoside binding (19-21).

The first protein discovered in this family was galectin-1 (LEG1; *LGALS1* gene, GenBank NM_002305), a small protein of 14 kDa that occurs as a monomer as well as a noncovalent homodimer of two single CRD subunits (19, 22). Galectin-1 can be secreted and, depending on the cell type or state of differentiation, it has been found in the nucleus, cytoplasm and ECM (23). Although galectins in general and galectin-1 in particular were first described as lectins that bind β-galactosides, it is now clear from the literature that in addition to being a lectin, galectin-1 is also engaged in protein–protein interactions. Interestingly enough, in most cases the lectin activity of galectin-1 is observed when it is extracellular, while the protein–protein interactions of galectin-1 concern its intracellular functions (23).

Multimodal activities of galectin-1 in the mechanisms of tumor progression

All the mechanisms discussed in the following sections are illustrated in Figure 1. It has been proposed that galectin-1 mediates cell adhesion and migration and is involved in several processes, including proliferation, apoptosis and even mRNA splicing (23). A series of studies in experimental models and cancer patients have reported significant correlations between the expression level of galectin-1 in tumor cells or adjacent tissues and tumorigenesis, metastatic potential and/or poor clinical outcome (20, 23, 24). Galectin-1 is a major player in glioma cell migration (25-27). Strong proof of the direct involvement of galectin-1 in tumor development and progression relates to its interaction with Ras for its activation, which induces and is required for tumor growth. Even if many genes are defective, correction of the Ras defect alone is sufficient to reverse the process (28). Galectin-1 acts as an escort protein for Ras plasma membrane anchorage and was recently shown to be both a structural component and a regulator of H-Ras nanoclusters (29-32).

1. Regulation of cell proliferation and resistance to apoptosis

Over the past few years, the role of galectin-1 in tumor growth has remained unclear. While the endogenous protein may function as a growth-promoting factor, exogenously added galectin-1 will not affect the growth of certain cell types, such as naïve T cells, astrocytic or colon cancer cells, but in contrast will specifically suppress tumor cell proliferation (23). Interestingly, it has been reported that galectin-1 exerts a biphasic modulation of cell growth. While high concentrations (~ 1 μM) of galectin-1 inhibit cell proliferation independent of its sugar-binding activity, low concentrations (~ 1 nM) are mitogenic and are susceptible to inhibition by lactose (33). Furthermore, galectin-1 can also regulate cell cycle progression in human tumor cells (34). The knockdown of galectin-1 in murine melanomas (35) or human glioma cells (our unpublished data) does not affect their growth rate in vitro, while it decreases the growth of 9L rat gliosarcoma (36). The seemingly paradoxical positive and negative effects of galectin-1 on cell growth are highly dependent on cell type and cell activation status, and may also be influenced by the relative distribution of monomeric versus dimeric or intracellular versus extracellular forms.

Moreover, the *O*-glycosylation status of cancer cell types seems to regulate their sensitivity to galectin-1-induced apoptosis. Valenzuela et al. (37) identified a glycosyltransferase, the core 2-*N*-acetylglucosaminyltransferase, which is downregulated in galectin-1-resistant human prostate cancer LNCaP cells compared to

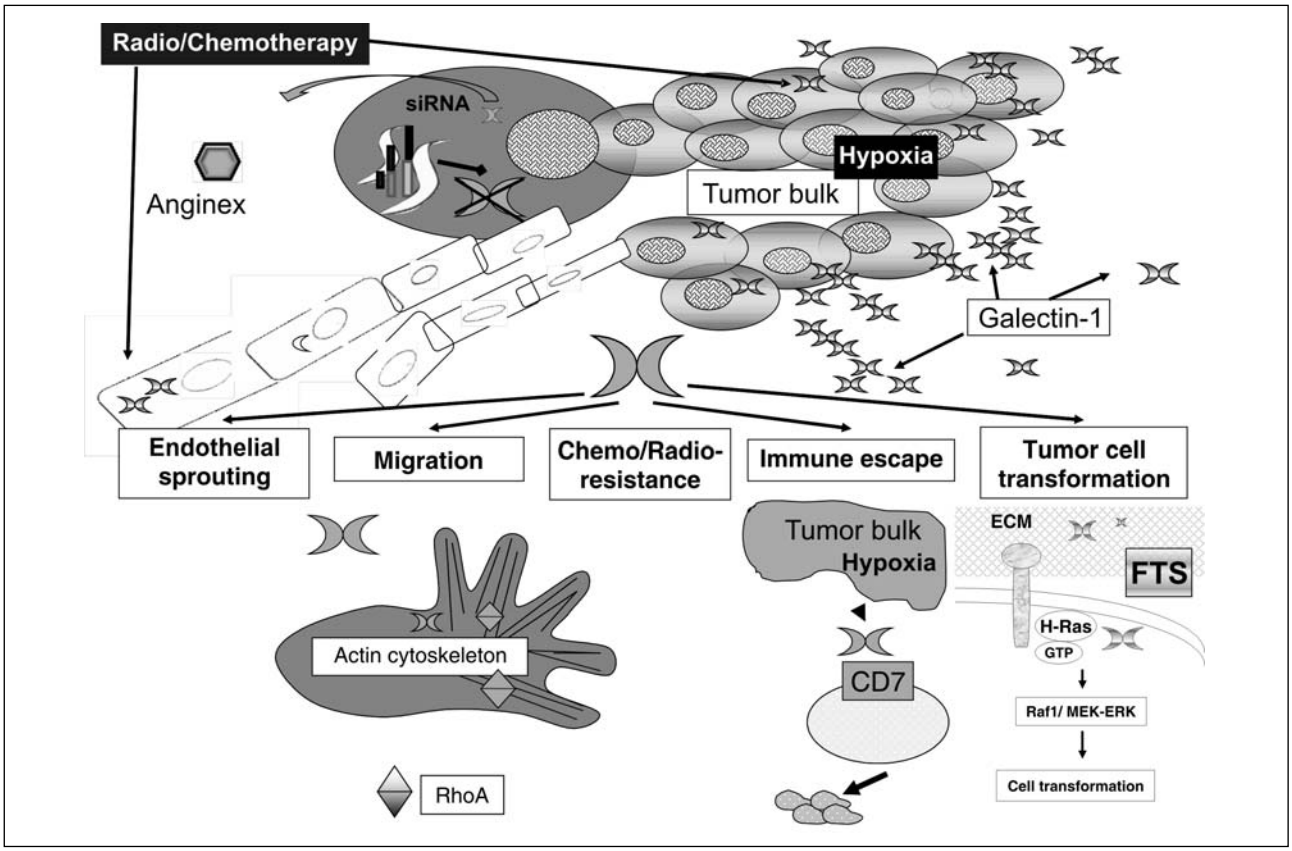

Fig. 1. Under stress conditions (black squares), such as hypoxia, radiotherapy and/or chemotherapy, the increase in galectin-1 expression in cancer cells, at least in head and neck squamous cell carcinoma (HNSCC), tumor astrocytes and melanoma, may activate their migration, increase resistance to radio- and/or chemotherapy, and promote tumor immune escape and angiogenesis. Intracellular galectin-1 could be targeted by small interfering RNA (siRNA) strategies. In contrast, extracellular galectin-1 could be the target of the angiogenesis inhibitor anginex and farnesylthiosalicylic acid (FTS, salirasib), a Ras farnesylcysteine mimetic. ECM, extracellular matrix.

galectin-1-sensitive LNCaP cells. Intriguingly, this is the same glycosyltransferase required for galectin-1 susceptibility of T lymphoma cells (38, 39), indicating that similar *O*-glycan ligands on different polypeptide backbones may be common death trigger receptors recognized by galectin-1 on different cancer cell types. Blocking *O*-glycan elongation by expressing α-2,3-sialyltransferase 1 rendered LNCaP cells resistant to galectin-1-induced apoptosis, showing that specific *O*-glycans are critical for galectin-1 susceptibility (37).

Galectin-1 is also negatively regulated by p53 (40), and reciprocally, it has been shown to modify p53 biological functions (27). p53 triggers apoptosis in response to cellular stress, including chemotherapy (41, 42), while loss of p53 functionality leads to chemoresistance (42). The reciprocal control exerted by galectin-1 or p53 on the other could be implicated in tumor chemoresistance, as could also be the case with respect to the interaction between Ras and galectin-1. Ras signaling and oncogenesis depend on the dynamic interplay of Ras with distinctive plasma membrane microdomains and various intracellular compartments. Such interactions are dictated by individual elements in the carboxy-terminal domain of the Ras proteins, among which one is recognized by galectin-1, galectin-3 and cGMP-specific phosphodiesterase δ. Galectin-1 thereby promotes H-Ras signaling to Raf at the expense of phosphatidylinositide 3-kinase (PI3K) and the Ral guanine nucleotide exchange factor (RalGEF), while galectin-3 promotes K-Ras signaling to both Raf and PI3K (29). When K-Ras-GTP interacts with galectin-3, it gains a conformation that promotes activation of Raf, PI3K and a third signal that attenuates ERK activation (30). Galectin-1 may therefore be involved in these Ras-related pathways implicated in tumor resistance to apoptosis (6, 43).

2. Regulation of cell migration

Cell migration is the net result of adhesion, motility and invasion (3, 5, 6), and galectin-1 modifies each of these three migration-related processes (23).

- *Adhesion:* Galectin-1 has been shown to increase the adhesion of various normal and cancer cells to the ECM via cross-linking of glycoproteins (integrins) present on cell surfaces with the carbohydrate moieties of ECM components such as laminin and fibronectin (44-46). In addition, galectin-1 can also mediate homotypical cell interactions, i.e., the aggregation of human

melanoma cells (47), and heterotypical cell interactions, such as those between cancer and endothelial cells, which in turn then favors the dispersion of tumor cells (48, 49).

- *Motility:* Galectin-1 increases the motility of glioma cells and induces actin cytoskeleton reorganization, associated with an increased expression of RhoA, which in turn modulates actin polymerization and depolymerization (26). Conversely, the knockdown of galectin-1 expression in glioma cells reduces motility and adhesiveness (26, 27). Oxidized galectin-1 stimulates the migration of Schwann cells from both the proximal and the distal stumps of transected nerves and promotes axonal regeneration after peripheral nerve injury (50). In contrast, in colon carcinomas, a galectin-1-enriched ECM decreases carcinoma cell motility (51).
- *Invasion:* Using a proteomic approach based on the comparison of highly and poorly invasive breast carcinoma cell lines, Harvey et al. identified the membrane expression of galectin-1 as a signature of cell invasiveness (52).

3. Galectin-1 and angiogenesis/hypoxia

An adequate vasculature is required for tumor growth. Therefore, the need for neovessel formation (or angiogenesis) provides a target for the treatment of cancer (53). Galectin-1 is overexpressed in endothelial cells of different human tumors (48, 54, 55). Moreover, Thijssen et al. (56) provided direct functional evidence that galectin-1 is required for tumor angiogenesis and the outgrowth of tumors.

Hypoxia helps confer cellular resistance to conventional chemotherapy and radiotherapy, modulates the unfolded protein response during endoplasmic reticulum stress (ERS) and accelerates malignant progression (57-59). Galectin-1, the expression of which is stimulated by hypoxia (59, 60), is a potent modulator of glioblastoma cell migration (26, 27, 61), but is also a proangiogenic molecule (56). Radiotherapy can increase galectin-1 expression in endothelial (62) and glioma cells (63), and subsequently activate glioma cell migration (64). Le Mercier et al. have recently shown that subtoxic doses of temozolomide, a drug routinely used against glioma, may favor the migration of certain glioma cells by increasing their levels of galectin-1 (14). The data suggest that increased galectin-1 expression in human glioma cells represents a defense mechanism against adverse effects, including chemotherapy and/or radiotherapy (62, 63), and also hypoxia (59, 60). Mathieu et al. observed the same features in melanoma cells (15).

Together these data suggest that in response to adverse conditions, such as hypoxia, radiotherapy and/or chemotherapy, activation of galectin-1 expression in cancer cells (14, 15, 59, 65, 66) does not appear to be associated with apoptosis because of their *O*-glycosylation status (37), but may perversely activate their migration (26, 27, 61), the unfolded protein response and angiogenesis (13, 56), and potentially enable them to move away from these adverse environmental conditions.

4. Galectin-1 involvement in tumor progression and tumor immune escape

An important survival strategy of tumor cells is the suppression of the tumor-specific immune response and evasion from T-cell attack. A number of mechanisms have been described that potentially contribute to this evasion of the antitumor immune response (17, 18). For example, galectin-1 functions as a homeostatic agent by modulating innate and adaptive immune responses. Galectin-1 induces cell cycle arrest and inhibition of cell growth (67, 68) and promotes the apoptosis of activated, but not resting, immune cells (69-71). Regulated glycosylation controls T-cell processes, including their activation, differentiation and homing, by creating or masking ligands of endogenous lectins. Differential glycosylation of Th1, Th2 and Th17 effector lymphocytes selectively regulates their susceptibility to galectin-1-induced cell death (72). This induced death of T cells relates to galectin-1 binding to CD7, CD43 and CD45 receptors on T cells (23, 73), inducing membrane lipid reorganization (74). An early cell response triggered by galectin-1 is the induction of tyrosine phosphorylation, a step that requires the presence of functional p56lck and ZAP-70 (75). Subsequently, ceramide is released, Bcl-2 is downregulated and the subsequent depolarization of the mitochondria is followed by caspase activation (first caspase-9 as the initiator caspase and then caspase-3 as the effector caspase) and breakdown of nuclear DNA (74). The specific expression of galectin-1 (which is considered an antiinflammatory protein) by the tumor-associated stromal cells (46, 76-79) or tumor-associated endothelial cells (in contrast to nontumor or stromal cells; 48), or a specific subpopulation of tumor cells (126), suggests that galectin-1 might trigger the death of infiltrating T cells and therefore protect these tumor sites from damage induced by T-cell-derived proinflammatory cytokines. Le et al. (59) and Saussez et al. (65, 66) have demonstrated a significant negative relationship in head and neck squamous cell cancers (HNSCCs) between galectin-1 expression and tumor T-cell infiltration. The direct involvement of galectin-1 in tumor immune escape processes has been elegantly demonstrated by Rubinstein et al. (35) in experimental melanomas and was further demonstrated in other types of tumors. Valenzuela et al. (37) found that galectin-1-expressing prostate cancer cells killed bound T cells, whereas LNCaP prostate cancer cells that do not express galectin-1 did not kill T cells. Moreover, prostate cancer cells further favor their survival and immune evasion by inducing the expression of galectin-1 by endothelial cells, which then inhibits T-cell transendothelial migration (80). Data collected by Juszczynski et al. (126) implicate galectin-1 produced by small numbers of neoplastic Reed-Sternberg cells in the development and maintenance of an immunosuppressive Th2/Treg-skewed microenvironment in classical Hodgkin's lymphomas and provide the molecular basis for selective galectin-1 expression in these Reed-Sternberg cells. Thus, galectin-1 appears to be a potential therapeutic target for restoring immune surveillance in lymphoma (81).

The direct involvement of galectin-1 as a hypoxia-regulated proangiogenic factor, an agent involved in cancer cell chemoresistance and possibly resistance to radiotherapy, a promigratory molecule for various cancer cell types, and a molecule markedly modulating tumor immune escape (Fig. 1) makes it a potentially interesting target in a number of invasive and/or metastatic cancers, especially gliomas, melanomas and HNSCCs.

Anti-galectin-1 compounds as potential anticancer drugs

Inhibitors targeting the carbohydrate recognition domain (CRD) of galectin-1

Galectins exhibit significant differences in glycan binding specificity but also show overlapping recognition of some glycans (82-85). Even slight modification to the basic LacNAc core impacts on glycan recognition by galectin-1 and suggests that unique subsites exist with each CRD. Modification to the LacNAc core can enhance, permit or preclude modified LacNAc recognition by each respective galectin (84). Although dimeric galectin-1 binds preferentially to glycoconjugates containing the ubiquitous disaccharide *N*-acetyl-lactosamine (Gal-β1–3/4 GlcNAc, also known as LacNAcII or type 2 saccharide), its binding to individual lactosamine units is characterized by relatively low affinity (K_d ~50 μM) (84, 86, 87). It is the arrangement of lactosamine disaccharides in multiantennary repeating chains (up to three branches) that increases the binding avidity (K_d ~4 μM) (86, 87). In contrast, there is no increase in avidity when the recognition unit is repeated in a string (poly-*N*-lactosamine) (87). In polysaccharides, galectin-1 does not bind glycans that lack a terminal nonreducing, unmodified *N*-acetyl-lactosamine (88, 89). Although terminal galactose residues are important for galectin-1 recognition, galectin-1 binds similarly to α3-sialylated (created by ST3Gal III sialyltransferase) and α2-fucosylated (created by fucosyltransferase) terminal *N*-acetyl-lactosamine, but not to α6-sialylated or α3-fucosylated terminal *N*-acetyl-lactosamine (84, 90, 91). Whether extended or otherwise, free ligands in solution bind galectin-1 with relatively low affinity (91). In contrast, the avidity of galectin-1 for extended glycans is enhanced when it is surface-bound and clustered as on cell surfaces or in the ECM (70, 91, 92).

The natural small saccharides, such as β-D-galactose, D-lactose and *N*-acetyl-lactosamine, bind to galectins and can inhibit their biological activity (26, 85, 93). Other small saccharides, such as β1,3glcNAc, galβ1,3Ara and galβ1,4Man, can inhibit galectins between two and four times more effectively than lactose (93, 94). However, all these small natural ligands have poor specificity towards any one galectin in particular. Although this class of saccharides is weakly specific and too sensitive to hydrolysis to permit any to be developed as drugs, Beuth et al. were among the first to show interest in the potential therapeutic benefits of galectin inhibitors. They showed that when administered i.p. every 8 h at 2 mg/g, D-galactose and arabinogalactan inhibited the liver metastasis of L-1 sarcoma cells in mice (95, 96).

The natural ligands of galectins are very complex polysaccharides which, as indicated previously, are too sensitive to hydrolysis and polar to be developed as drugs. To overcome these limitations, strategies to develop less polar, competitive inhibitors of the CRD of galectin-1 have been pursued by several groups (97). We will focus here on strategies with proven anti-galectin-1 activity. However, given that galectins exhibit overlapping recognition of certain glycans, only a limited number are exclusively specific for galectin-1.

Methylation of the anomeric function of the natural ligands of galectin-1 revealed that this single modification gave enhanced inhibitory effects. One such derivative, 1-methyl-β-D-lactoside (**1;** Fig. 2), although not fully specific, is considered to be the benchmark inhibitor of galectin-1, as it binds to its CRD with similar affinity (K_d ~190 μM) to that of natural ligands and thus competes with natural ligand binding (98). The potential therapeutic benefit of such anti-galectin-1 derivatives was indicated by Oguchi et al. (99) in the very aggressive B16 murine melanoma model, in which the development of lung metastases was reduced by 35-45%. This methylation of the anomeric function was also undertaken with *N*-acetyl-lactosamine, another natural disaccharide. Its 1-methyl derivative (**2**; Fig. 2) also gave good in vitro inhibitory potency against galectin-1 and galectin-3 (K_d = 70 and 67 μM, respectively) (100).

Thiogalactosides and thiolactosides were then synthesized as specific inhibitors of human galectin-1 and -3 (101). The two thiolactoside derivatives **3** and **4** (Fig. 2) revealed good inhibitory properties, with K_d values between 40 and 80 μM, although a thiogalactoside derivative appeared to be less potent, as it was found to have lower binding affinity (K_d ~ 2.5 mM). The inhibitory potency of the thio derivatives of these sugars is 20 times higher than that of galactose or lactose themselves. This enhancement of inhibitory potency of thio derivatives is specific to galectin-1 and -3 and was more pronounced against the former than the latter (101). Thiodigalactoside **5** (Fig. 2), another thio derivative, was shown to bind to the CRD of galectin-1 (102, 103) and found to impair the in vitro proliferation of rat hepatic stellate cells by inhibiting the biological activities of both galectin-1 and -3 (104).

Because x-ray crystal structures of the carbohydrate recognition domain of human galectin-3 with *N*-acetyl-lactosamine show extended binding sites close to the 3-OH function of the galactose residue, new LacNAc methyl glycosides with modifications on the galactose 3-OH function were synthesized to create additional favorable interactions with the protein and so permit the development of new high-affinity galectin-3 inhibitors (97, 105, 106). Although x-ray structures of the CRD of galectin-1 with LacNac did not show this extended pocket, the new C'3-thioureido derivatives of LacNAc **6** and **7** (Fig. 3) revealed affinities 3 times higher (K_d = 23 μM) than the LacNAc glycoside (107).

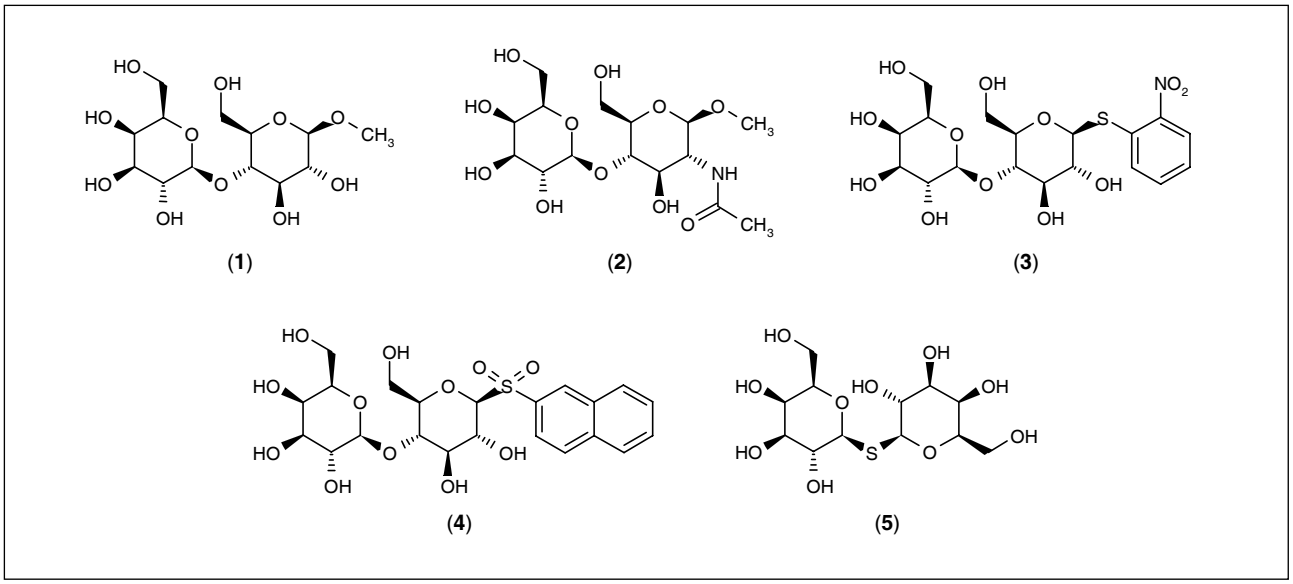

Fig. 2. Analogues of natural ligands with modified anomeric functions.

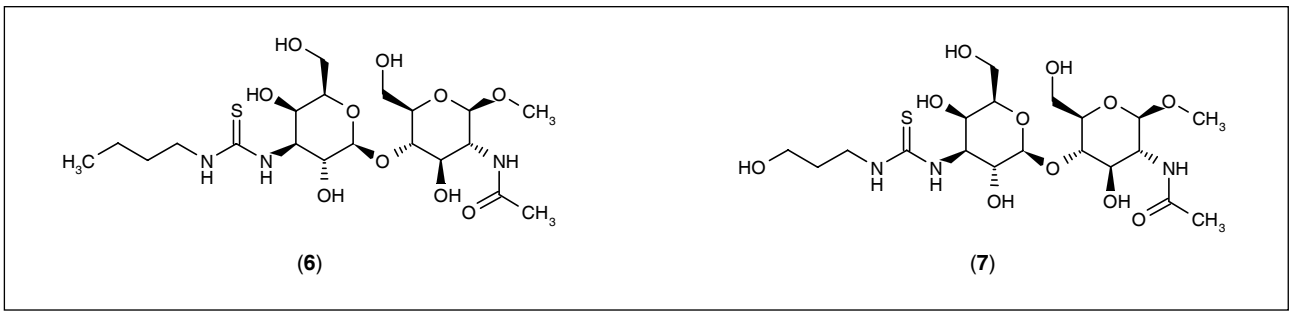

Fig. 3. Analogues of natural ligands with modified C'3-OH functions.

Several research groups have exploited the idea of using multivalent compounds to discover high-affinity galectin inhibitors. Nilsson's group has developed new scaffolds between the linked carbohydrate units based on polyfunctional unnatural amino acids like phenyl-bis-alanine (PBA) and phenyl-tris-alanine (PTA). One divalent and one trivalent lactoside derivative (**8** and **9**, respectively; Fig. 4) revealed good affinity towards galectin-1, with a dissociation constant in the range of 7-8 μM. Another interesting derivative, the divalent lactoside **10** (Fig. 4), not only has strong affinity (3.2 μM) but also relatively good specificity for galectin-1 (98).

Glycodendrimers offer the possibility of synthesizing high-affinity ligands for sugar receptors like galectins, as these types of structures offer a high density of carbohydrate ligands on their surface. Thus, the probability of sugar receptors finding another carbohydrate ligand in the immediate proximity is increased (97). Gabius and Pieters' group developed this concept to obtain anti-galectin products. The glycodendrimer **11** (Fig. 5), generated by using 2,3-di-(2-aminoethoxy)benzoic acid as the starting material, has 1,500 times higher potential for galectin-1 inhibition than free lactose (108, 109). Six further generations of lactose dendrimers revealed potent inhibition of galectin-1 and -3 (IC_{50} = 0.1-124 μM) compared to free lactose (up to 14,000 times more potent) (110). This enhanced inhibition is also more pronounced against galectin-1 than against galectin-3. Although these compounds seem to be well tolerated by mammalian cells and small rodents (111), to date there is no proof of any in vivo activity.

A large number of natural products with steroid backbones discovered in various sponge species display antimigratory and cytotoxic effects, including the lactosylated steroids. One of these compounds (**12**; Fig. 6) significantly modified the in vitro migration of human U-373 MG glioblastoma and PC-3 prostate cancer cells. The mono-β-lactosylated derivative **12** revealed in vivo activity in human U-373 MG glioblastoma and mouse lymphoma models (93) related to the binding of its lactoside moiety to the galectins involved in cancer cell migration (6, 93). Mono-β-lactosylated **12** displays a binding affinity for galectins in the mM range, while its bi-β-lactosylated analogue **13** (Fig. 6) displays a binding affinity in the μM range (93, 97).

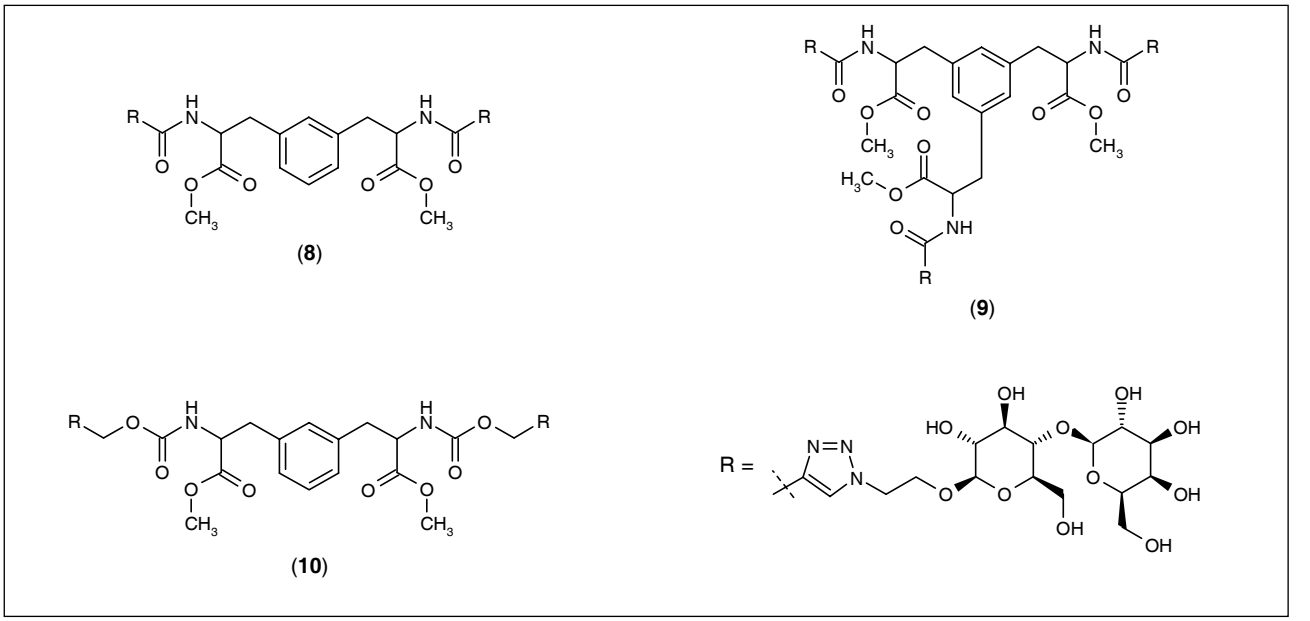

Fig. 4. Multivalent lactose derivatives that selectively inhibit galectin-1.

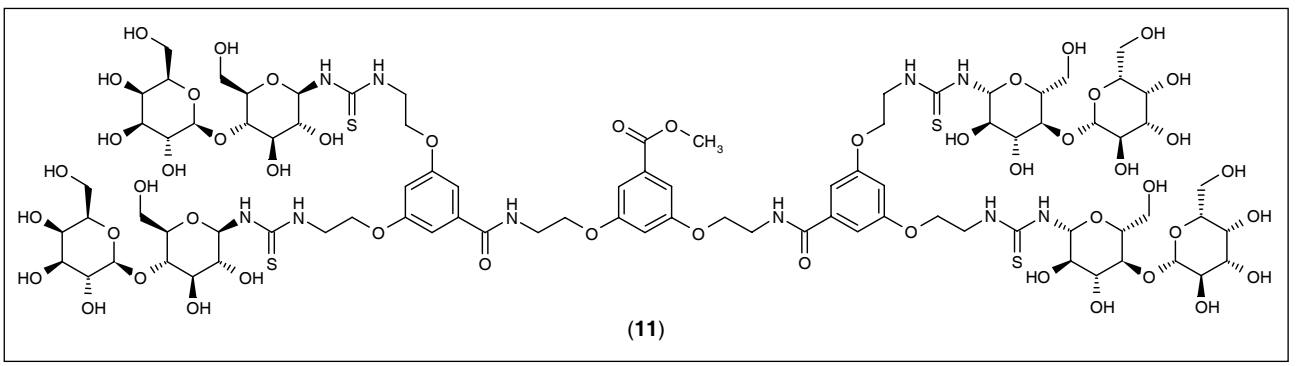

Fig. 5. A dendrimer with potent inhibitory activity against galectin-1.

Peptides and antibodies neutralizing the activity of galectin-1

Proteins and peptides selected experimentally for high-affinity interactions with predetermined target structures are emerging as important molecules that could serve to extend conventional drug therapy (112). Considerable progress has been made in recent years to convert peptides into therapeutically useful molecules, particularly for the targeting of cell-surface molecules (113-115). This resulted in the development of *short synthetic peptides* that bind to the galectin-1 CRD with a high level of affinity and specificity. This therefore offers an attractive approach to inhibiting the function of galectin-1- and β-galactoside-mediated cell adhesion. Although there are no descriptions currently available concerning clinical trials with galectin-1-targeting antibodies or peptides, the development of such approaches to interfere with galectin-1 oligomerization or the interaction of galectins with their ligands has already been described at the in vivo preclinical phase and demonstrates the potential feasibility of the approach. As early as 1986, Meromski et al. highlighted the fact that a monoclonal antibody directed against endogenous galactoside-specific lectins was able to inhibit homotypic melanoma and fibrosarcoma cell aggregation and adhesion, and to reduce the colonization of the lungs by these cancer cells in mice (116).

Topomimetics of the antiangiogenic peptide anginex

Since they are easily accessible to agents delivered by the blood, endothelial cells that line the tumor vasculature are particularly pertinent target cells for therapeutic approaches (117). However, to affect only tumor vasculature, specific targets on angiogenically active endothelial cells are essential. To date, only a few targets on tumor vasculature have been identified. Thijssen et al. (56) showed that galectin-1 is a receptor for the angiogenesis-inhibitory peptide anginex and consequently could be a

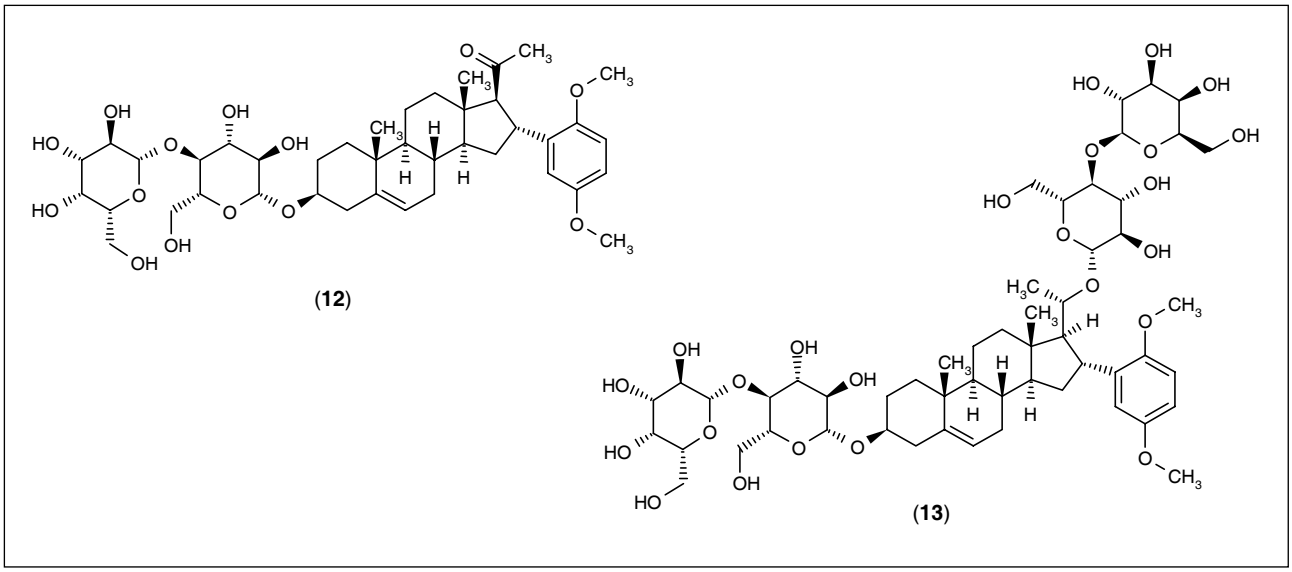

Fig. 6. Lactosylated steroids with anti-galectin activity.

crucial protein for tumor angiogenesis. Nonpeptidic calixarene-based protein-surface topomimetics of the antiangiogenic peptide anginex are potent angiogenesis inhibitors and highly effective in inhibiting tumor angiogenesis and growth in mouse models (MA148 human ovarian carcinoma and B16 murine melanoma) (118). Furthermore, this group demonstrated that this type of peptide synergistically improves chemotherapy and radiosensitizes endothelial cells (119, 120).

Approaches for impairing Ras activation

One way to block Ras protein activity is by interfering with its spatiotemporal localization in cellular membranes or in membrane microdomains, a prerequisite for Ras signaling and biological activity (28). As explained above, galectin-1 interacts in a lactose-independent manner with H-Ras-guanosine triphosphate (H-Ras-GTP) through its farnesylcysteine carboxymethylester (31, 32) and so strengthens its membrane association (31). Galectin-1/Ras interactions are directed by the GDP/GTP loading state of Ras, as well as by the *C*-terminal polybasic farnesyl domain in K-Ras 4B and the cysteine-palmitoylated *C*-terminal farnesyl domains in H-Ras and N-Ras (the K-Ras 4B isoform has no palmitoylated cysteines). Farnesylthiosalicylic acid (FTS, salirasib) is a Ras farnesylcysteine mimetic that selectively disrupts the association of chronically active Ras proteins with the plasma membrane. FTS competes with Ras for binding to Ras escort proteins, which possess putative farnesyl-binding domains, like galectin-1, and interact only with the activated form of Ras proteins, thereby promoting Ras nanoclusterization in the plasma membrane and robust signals. Phase I clinical trials have demonstrated a good safety profile for oral FTS, with minimal side effects and promising activity in hematological malignancies (28).

Sequence-specific knockdown of galectin-1

The specific knockdown of gene expression can be achieved by various approaches, including, for example, antisense oligodeoxynucleotides (ODNs) and small double-stranded interfering RNAs (siRNAs), both of which are being widely investigated therapeutically. These approaches mostly work at posttranscriptional levels and rely on the transfection into cells of either antisense ODNs or siRNA molecules that hybridize exclusively with the mRNA of the targeted gene and block the synthesis of the corresponding protein (121, 122). As detailed in the next section, siRNA-induced knockdown of galectin-1 expression significantly improves the in vivo response to the cytotoxic drug temolozomide in orthotopic human melanoma and glioma xenograft models (13-15).

Proof of concept of anti-galectin-1 molecules as potential anticancer drugs

Major findings obtained with respect to experimental anti-galectin approaches in various in vivo models of murine and human cancers are summarized in Table I. The therapeutic benefits of anti-galectin-1 compounds have already been proven in vivo in orthotopic models of gliomas (14) and melanomas (15). Furthermore, the in vitro transient transfection of an siRNA directed against galectin-1 in an invasive human glioma cell line prior to its in vivo intracranial grafting into the brain of nude mice significantly improved the survival of these animals compared to controls. This siRNA-induced decrease in galectin-1 expression also significantly improved the response to the pro-autophagic drug temolozomide by impairing the capabilities of glioma cells to respond to ERS (14), which is directly involved in resistance to chemotherapy (123, 124) and is activated during hypoxia (58, 125). We have also succeeded in increasing the sur-

Table I: Major findings obtained with respect to experimental anti-galectin approaches in various in vivo models of murine and human cancers

Cancer	Treatment	Results	Ref.
Human glioblastoma (Hs 683)	Small interfering RNA directed against galectin-1 in combination with the cytotoxic drug temozolomide	Significant increase in the survival of brain tumor-bearing mice; increased sensitivity to chemotherapeutic drugs	13, 14, 123
Murine lung sarcoma (L-1)	D-Galactose (**1**) (2 mg/g i.p. for 3 days)	Significant decrease in liver metastases	95, 96
Murine melanoma (B16)	1-Methyl- -D-lactoside (8) (pretreatment of tumor cells before in vivo inoculation)	Significant decrease in lung metastases	99
Murine melanoma (B16)	Small interfering RNA directed against galectin-1 in combination with the cytotoxic drug temozolomide	Significant increase in the survival of tumor-bearing mice; sensitize tumor cells to chemotherapeutic drugs	15
Murine melanoma (B16)	Targeted inhibition of galectin-1 gene expression by stable transfection with oligodeoxy-nucleotide prior to grafting	Heightened T-cell-mediated rejection	35
Human glioblastoma (U-373 MG) and murine lymphoma (P388)	Lactosylated steroid injected i.v. 3 times a week for 5 weeks at 40 mg/kg	Significant increase in the survival of tumor -bearing mice; the U-373 MG model is orthotopically grafted into the brain of nude mice; the s.c. P388 lymphoma model metastasizes to the liver	97

vival of orthotopic human glioblastoma xenograft-bearing mice by direct in vivo localized delivery of siRNA against galectin-1 (into the third ventricle of the brain using s.c.-implanted micropumps). Delivery into the third ventricle was paralleled by stereotactic injections of the siRNA against galectin-1 into the intracranial tumor. This in vivo siRNA-induced decrease in galectin-1 expression in orthotopic human glioblastoma xenografts again increased the therapeutic benefits of temozolomide and a proapoptotic combination (procarbazine–lomustine–vincristine) (13, 126). Similar experiments showed the same therapeutic benefits in experimental models of melanoma (15). In the same manner, we have demonstrated a significant therapeutic benefit for a potential ligand of galectins, a lactosylated steroid, in aggressive models of human glioblastoma and mouse metastatic lymphoma (97). Other chemical templates have also previously been described as effective anti-galectin-1 compounds (93).

Conclusions

The direct involvement of galectin-1 as a hypoxia-regulated proangiogenic factor, a promigratory molecule for various cancer cell types, an agent involved in cancer cell chemoresistance and possibly radiotherapy resistance, and a molecule markedly modulating tumor immune escape should make galectin-1 a valuable target to combat different invasive and/or metastatic cancers, especially gliomas, melanomas and HNSCCs. Targeting galectin-1 by means of specific antibodies, antisense oligonucleotides, siRNAs or selective organic chemical molecules should reduce tumor immune escape, cancer cell migration and tumor neoangiogenesis and restore a certain level of chemo- and/or radiosensitivity in metastatic cancers.

Acknowledgements

We apologize to the many authors whose papers could not be cited owing to space limitations. We thank Dr. Vincenzo Facchini for his critical review of the manuscript. Robert Kiss and Florence Lefranc are Research Director and Clinical Research Fellow, respectively, with the Fonds National de la Recherche Scientifique (FNRS, Belgium). Véronique Mathieu is a postdoctoral researcher supported by the Fonds Yvonne Boël (Brussels, Belgium). Marie Le Mercier is the holder of a “Grant Télévie” (FNRS, Belgium). None of the authors declare any potential conflicts of interest.

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