REVIEW ARTICLE

New Roles for Galectins in Brain Tumors—From Prognostic Markers to Therapeutic Targets

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Despite advances in diagnosis and treatment, brain tumors continue to be the leading cause of cancer-related death in patients under 35 years of age, demonstrating the need for better prognostic and therapeutic targets. Galectins, a family of mammalian carbohydrate binding proteins, are involved in many processes important for tumor survival and dissemination, including proliferation, apoptosis, transcriptional regulation, intracellular signaling, cell adhesion, and cell migration. Several galectins are expressed in human brain, with many galectins demonstrating altered expression during tumor progression. Thus, galectins and the functions regulated by this family of proteins are potential targets for the diagnosis and treatment of brain cancer. This review highlights the roles of galectins in cancer and specifically, the developing field of galectins in brain cancer.

Brain Pathol 2005;15:124-132.

NEW DIRECTIONS IN THE STUDY OF BRAIN TUMORS

The diagnosis and treatment of brain tumors is undergoing a paradigm shift. New molecular approaches and model systems are enabling the identification of genes, proteins and signaling pathways that drive tumorigenesis and progression. Not surprisingly, many of these genes, proteins and signaling pathways also regulate normal brain development and formation, but are altered or aberrant in brain cancer. Since many of the signaling pathways that promote tumor formation and progression are also essential for tumor cell survival, drugs that specifically block these pathways are attractive targets for new anti-cancer drugs, providing a mechanism for selective elimination of neoplastic cells. Thus, considerable effort in brain tumor research is now directed towards identifying critical signaling pathways and their components, to define new molecular targets for therapy (32, 56, 60, 100).

The galectins, a family of proteins that recognize the sugar residues that decorate cellular glycoproteins or glycolipids, are an example of molecules that may play critical roles in tumorigenesis, tumor progression and tumor survival. Galectins have already been shown to play a key role in linking extracellular and intercellular signals to control survival and motility in lymphocytes and epithelial cells (31, 36, 79). In fact, roles for galectins in normal epithelial and hematopoietic development, as well as in epithelial carcinogenesis and lymphomagenesis, have been clearly demonstrated (35, 43, 44, 55, 61, 73, 80, 101). However, the roles of galectins in brain development, and in the development of brain tumors, are only beginning to be appreciated. By directly regulating the balance between cell survival and cell death, modulating cell motility, and impacting signaling through the Ras pathway, galectins may play important roles in the development and the potential treatment of brain tumors (31, 36, 70). In addition, by regulating immune responses, galectins may also determine the efficacy in brain tumor patients to a variety of experimental immuno- and vaccine-based therapies (31, 79). Thus, a wider appreciation of the role of galectins and their complexities in brain tumors is warranted.

WHAT ARE GALECTINS?

The galectins are a phylogenetically conserved family of lectins, proteins that recognize the sugar residues that decorate cellular glycoproteins or glycolipids. Galectins are expressed in species ranging from multicellular fungi and sponges, to insects, fish, birds and mammals. Fifteen galectins have been identified in mammals, and are expressed in a variety of tissues (17, 26, 91, 97, 105). All galectins share a core structure called the carbohydrate recognition domain (CRD) that recognizes and binds to sugar ligands (Figure 1). While the CRDs of all galectins share affinity for the minimal saccharide ligand N-acetyllactosamine, a common disaccharide found on many cellular glycoproteins, individual galectins can also recognize different modifications of this minimal saccharide ligand, demonstrating the fine specificity of certain galectins for tissue- or developmentally-specific ligands (2, 9).

In addition to their phylogenetic conservation, galectins have an overwhelming variety of essential functions. Galectins regulate cell cycle progression and apoptosis, serve as adhesion and deadhesion molecules, and are involved in RNA splicing (3, 5, 17, 43, 64, 68, 101). Numerous groups have noted the proliferative effects of several galectins (40, 64, 104). As early as 1987, Moutsatsos and colleagues noted an increase in galectin-3 expression in dividing 3T3 cells just before onset of S phase, suggesting that galectin-3 may be related to the proliferative state of cells (64). This hypothesis was supported by the demonstration that overexpression of galectin-3 in a T-cell lymphoma line conferred a growth advantage to the cells (104), and that galectin-3 expression was induced following mitogenic stimuli in T-cells; in contrast, expression of galectin-3 antisense cDNA in T-cells blocked galectin-3 expression and inhibited T-cell proliferative responses to mitogens (40).

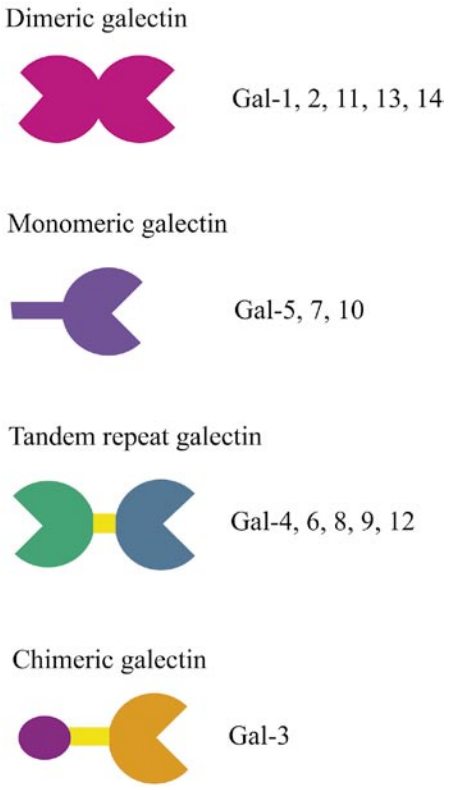

Figure 1. Schematic of the structures of galectin family members. Galectins can be divided into 3 major subgroups, the prototype, tandem repeat, and chimeric galectins. The prototype galectins contain one CRD and include galectins-1, 2, 5, 7, 10, 11, and 13. Many of these form homodimers that are able to cross-link identical ligands on the cell surface or the extracellular matrix. The tandem repeat galectins comprise 2 different CRDs connected by a short linker and include galectins-4, 6, 8, 9, and 12. The CRDs of the tandem repeat galectins can be connected by linker peptides of variable length, and have the potential to cross-link two different carbohydrate ligands. One chimeric galectin, galectin-3 has been identified in mammals. Galectin-3 has a single C-terminal CRD joined to a unique N-terminal domain important for the formation of higher order oligomers.

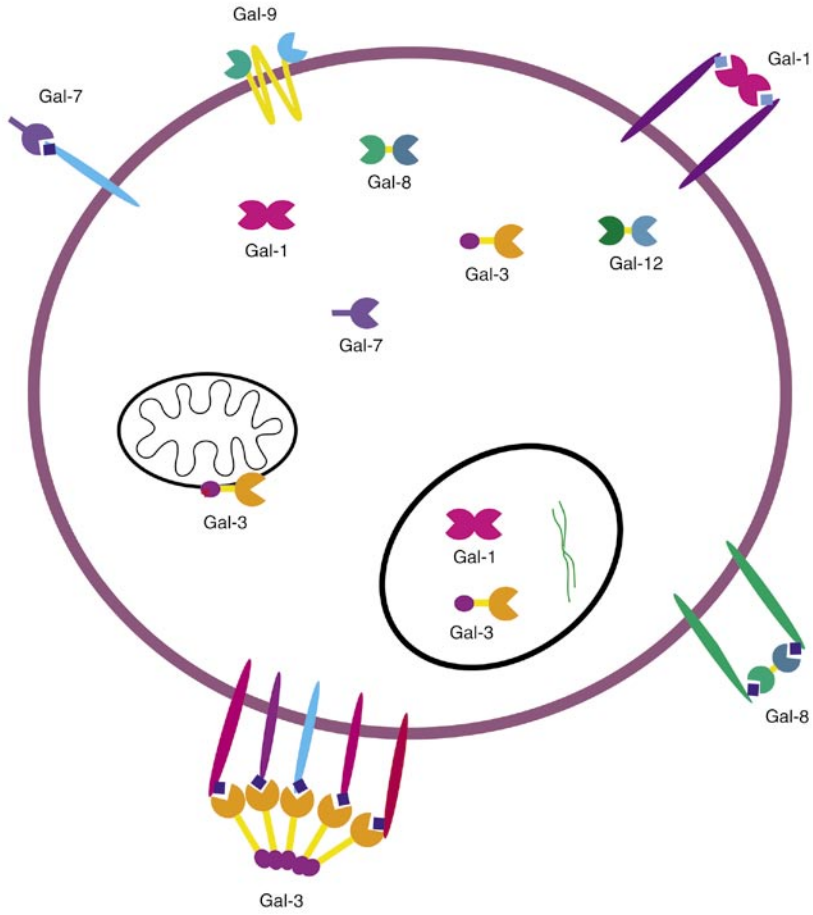

Figure 2. Diagram of intracellular and cell surface localization of several galectin family members. All galectins are produced in the cytosol. Galectins-1, 3, 7, 8, and 12 are all localized in the cytoplasm. Galectins-1 and 3 can also be transported to the nucleus, with roles in mRNA splicing and transcriptional regulation. In addition, galectin-3 can associate with the mitochondria following apoptotic stimuli. Galectins-1, 3, 7, 8, and 9 are secreted by a non-classical pathway. Once secreted, galectins avidly bind to glycoprotein or glycolipid ligands expressed on the cell surface or in the surrounding extra-cellular matrix. In addition, galectin-9 can insert into the plasma membrane, where it acts as a urate transporter in renal epithelial cells. The functions of galectins are influenced by the sites of localization inside or outside the cell.

Unlike other proteins that are typically targeted to a single cellular location, galectins are found at many sites within and outside the cell (Figure 2). Galectins are produced as cytosolic proteins, but can localize to the nucleus or associate with mitochondrial membranes. In addition, galectin-9 can insert into the plasma membrane where it acts as a urate transporter in renal epithelial cells (53). One compartment from which galectins are conspicuously absent is the ER-Golgi. As glycosylation of most secreted proteins occurs in the ER-Golgi, galectins may be excluded from this compartment in order to prevent disruption of processing and transport of nascent glycoproteins that would result from galectin-carbohydrate binding. Although galectins lack an N-terminal secretion signal and are excluded from the ER-Golgi secretory compartment, galectins are secreted from cells via a non-classical secretory pathway (16). Upon secretion, galectins typically bind to and stay associated with glycoproteins and glycolipids on the cell surface, or within the surrounding extracellular matrix (ECM). Thus, while galectins are abundant in tissues where they are made, only low levels are found in serum, and serum levels of galectins do not reflect the rates of synthesis in tissues (4, 30, 39).

THE MANY FUNCTIONS OF GALECTINS—WHERE THEY ARE DICTATES WHAT THEY DO

It is becoming increasingly clear that the function of a particular galectin depends on the localization of the protein within or outside the cell. For example, while galectin-3 expression increases in proliferating 3T3 cells, the level of cytoplasmic galectin-3 does not change with proliferation, only the level of nuclear galectin-3 increases (64). In prostate cancer cells, localization of galectin-3 in the cytosol promoted invasion, anchorage-independent growth, and angiogenesis, and inhibited apoptosis in response to apoptotic triggers. In contrast, localization of galectin-3 to the nuclei of the same prostate cancer cell line had the opposite effect, inhibiting invasion, reducing anchorage dependent growth and promoting apoptosis (11).

Within the nucleus, galectins-1 and 3 can act as pre-mRNA splicing factors via interactions with gemin4, a component

of the SMN splicing complex (68). Interestingly, the SMN gene was identified as the target gene in spinal muscular atrophy (SMA), and mutations in SMN may lead to reduced levels of proteins necessary for growth and function of motor neurons (71). Galectins may thus regulate protein production via control of mRNA processing, and disruption of galectin interactions with the nuclear splicing machinery may contribute to a variety of pathologic processes.

More recently, galectin-3 has been reported to regulate transcription. Galectin-3 interacts with and augments the transcriptional activity of the thyroid specific transcription factor (TTF-1) with implications for thyroid cancer, as TTF-1 is involved in the differentiation and proliferative potential of thyroid cells (69, 84). As TTF-1 belongs to a family of highly conserved transcription factors, galectin-3 may regulate transcription in other cell types as well (69).

Outside the nucleus, several galectins regulate the balance between cell survival and cell death. As mentioned above, intracellular galectin-3 confers a survival or proliferative advantage to many cell types. Specifically, cytoplasmic galectin-3 associates with the apoptotic regulatory protein Bcl-2 at mitochondrial membranes and blocks cytochrome c release, a key step in apoptosis (63). The cytoprotective effect of intracellular galectin-3 has been demonstrated in response to a plethora of cellular insults, including Fas cross-linking, UVB irradiation, staurosporine, nitric oxide, and anoikis (43, 52, 63, 104). In contrast to the anti-apoptotic function of intracellular galectin-3, intracellular galectin-7 expression in damaged keratinocytes sensitized the cells to UV induced apoptosis; the accelerated loss of damaged keratinocytes may be a mechanism preventing development of skin cancer (6).

In addition to regulation of apoptosis by intracellular galectins, several extracellular galectins directly induce apoptosis. Galectins-1, 2, 3, 7, and 9 all induce apoptosis of thymocytes, activated T lymphocytes, or human leukemic T-cell lines (22, 72, 73, 92, 96, 98). Galectin-1 also induces apoptosis of breast and prostate cancer cell lines (20, 101). Furthermore, galectin-9 induces apoptosis of melanoma cells and galectin-9 expression in primary melanoma lesions is associated with good prognosis in melanoma patients (42).

Other extracellular functions of galectins include regulating both adhesive and deadhesive interactions between cells or between cells and the ECM. For example, galectin-1 caused detachment of myoblasts and human vascular smooth muscle cells from laminin (27, 62), while galectin-1 promoted adhesion of CHO cells, mouse F9 cells, human ovarian carcinoma cells and human melanoma cells to laminin (90, 94, 107). These differences in adhesive versus de-adhesive functions may be related not only to the specific cell type involved, but also to the local galectin concentration. As shown in Figure 1, some galectins are monomeric, while others are dimeric or bivalent; multimeric galectins may be effective cross-linkers between saccharide ligands on the cell surface and within the ECM, while monomeric galectins may block ligands and promote deadhesion. As galectin-3 pentamerizes upon binding multivalent ligands (Figure 2), galectin-3 may be a very effective cross-linker of cells and matrix (1).

Modulation of cellular adhesion has direct effects on cell growth regulation, with many cell types requiring anchorage for survival and growth. Breast carcinoma cells expressing high levels of galectin-3 had increased adhesion to various ECM proteins compared to breast carcinoma cells expressing low levels of galectin-3 (57). The increased adhesion mediated by galectin-3 conferred a survival advantage to the breast cancer cells, with increased cell survival following apoptotic stimuli. Conversely, galectin-8 appears to be deadhesive for human lung carcinoma cells; addition of galectin-8 to the carcinoma cells prior to seeding in tissue culture reduced cell adhesion and increased susceptibility to apoptotic stimuli (28).

WHAT ROLES DO GALECTINS PLAY IN CANCER?

As suggested by the various galectin functions described above, galectins play key roles in tumorigenesis and cancer progression. Aberrant galectin expression has been demonstrated in many different types of cancer. While a small number of studies suggest a tumor-suppressive role for some galectins, most studies demonstrate galectin cancer-promoting activity. Accordingly, expression of several different galectins is often higher in cancerous compared to normal tissue.

Since galectin-3 is anti-apoptotic in many cell types, galectin-3 expression in tumors is often associated with poor prognosis. Galectin-3 has been suggested as a diagnostic and prognostic marker for thyroid cancer (15, 21, 38, 86) and is required for the highly proliferating transformed phenotype of thyroid papillary carcinoma cells (106). Galectin-3 has also been described as a prognostic marker for colon cancer (65, 89) and there are reports of increased galectin-3 expression in other cancers including head and neck squamous cell, pancreas, bladder, stomach, and renal carcinomas (93).

Galectin-3 can promote malignancy through a variety of mechanisms. As mentioned above, intracellular galectin-3 can directly associate with mitochondrial membranes, block release of pro-apoptotic factors, and prevent apoptosis, as shown in breast cancer (63). Intracellular galectin-3 expression is also high in aggressive large B-cell lymphomas, and protects B lymphoma cells against apoptosis (35). Increased levels of galectin-3 may also affect mRNA processing and transcription, as noted above, resulting in altered cellular regulation of protein production and turnover, and thus contribute to tumorigenesis (68).

Like galectin-3, galectin-1 can participate in pre-mRNA processing (68). Intracellular galectin-1 has additional tumor-promoting activities, as galectin-1 is involved in membrane anchorage of oncogenic Ras, a key step in Ras-induced cellular transformation (70). As the structures of both galectin-1 and Ras are known (10, 54), this pathway may be an attractive target for molecular inhibitors to disrupt growth and survival of transformed cells.

Outside the cell, galectins participate in cellular adhesion, promoting survival for anchorage dependent cells, as well as facilitating invasion of neighboring tissue and migration to distal sites (51, 57). As described above, breast carcinoma cells that express high levels of galectin-3 bound avidly to and spread rapidly on extracellular matrix (ECM) proteins, while the same breast carcinoma cell line expressing relatively little or no galectin-3 spread poorly (57, 99). Expression of galectin-3 antisense cDNA in a highly malignant breast cancer cell line restored cell contact-mediated

growth inhibition and abrogated serum-independent and anchorage-independent growth in vitro. In addition, inhibition of galectin-3 expression led to suppression of tumor growth in nude mice (33).

Expression of some galectins may also protect tumors from immune attack. The immune system is responsible for removal of foreign cells; many tumors may appear "foreign," as tumor cells can express neoantigens that are recognized by CD8 T-cells (22). However, as galectins-1, -3, and -9 can directly kill T-cells, expression of these galectins on the tumor cell surface could thwart the immune response to the tumor. This mechanism of tumor immune evasion has been directly demonstrated for galectin-1 in a murine melanoma model. Inhibition of galectin-1 expression by the melanoma cells in vivo resulted in increased CD8 T-cell mediated immunity to the tumor and tumor rejection, apparently by increasing the survival of CD8 T-cells that attacked the tumor cells (85). As increased galectin-1 expression is found in many types of cancer, including bladder, prostate, ovary, thyroid, and endometrium, compared to non-transformed cells (93), galectin-1 on the surface of these types of tumor cells may also kill infiltrating T-cells.

In addition to increased galectin-1 expression on the surface of tumor cells, galectin-1 expression is increased in the stromal tissue surrounding tumors in prostate, ovary, and head and neck carcinomas, compared to normal tissues (24, 95). Increased deposition of galectin-1 in tumor-associated stroma is associated with tumor progression, and galectin-1 presented on ECM can directly kill adherent T-cells (30, 88, 95). Thus, galectin-1 in tumor stroma may both promote adhesive interactions required for tumor cell migration and metastasis, and also protect tumors from immune attack (30).

WHAT ROLE DO GALECTINS PLAY IN BRAIN CANCER?

Galectin-1 and galectin-3 are highly expressed in brain (7, 14, 18, 25, 74, 75) In human brain, galectin-3 is expressed by fibroblasts, macrophages, activated microglial cells, a subpopulation of dorsal root ganglia neurons, and Schwann cells following nerve injury (29, 75, 76, 82, 102). Galectin-1 expression has not been well-characterized in human brain; however, in animal models, galectin-1 is expressed in a subpopulation of dorsal root ganglia neurons, primary sensory neurons, and motor neurons as well as astrocytes, perivascular cells, and microvessels (37, 41, 55, 81, 87). The functions of galectin-3 and galectin-1 in normal brain remain largely unknown. No neural developmental defects have been described for galectin-3 knockout mice. However, galectin-1 knockout mice demonstrate defects in olfactory neuron targeting (78). Consistent with the findings in the knockout mice, galectin-1 has recently been shown to stimulate axonal regeneration (34, 58, 59).

Most studies of galectins in brain cancer have focused on galectin-1 and galectin-3. Galectin-1 expression in brain cancer is associated with malignancy and poor prognosis. Increased expression of galectin-1 mRNA and immunoreactive galectin-1 protein correlated with increased malignancy in a study of 27 astrocytic tumors, ranging from low-grade astrocytoma to malignant glioma (103). In this study, transfection of glioma cell lines with galectin-1 antisense cDNA reduced cell proliferation and inhibited anchorage-independent cell growth. In a larger study, Rorive et al. performed immunohistochemical analysis of galectin-1 expression in 220 tumors, including 151 astrocytic, 38 oligodendroglial, and 31 ependymal tumors. In this study, all gliomas expressed galectin-1. However, higher levels of galectin-1 were observed in high-grade astrocytic tumors with patient survival less than 12 months, compared to lower levels of galectin-1 in tumors from patients with long survival periods (over 48 months). To examine potential mechanisms responsible for shorter survival in patients with tumors expressing high levels of galectin-1, this group examined cultured human glioblastoma cells and found that addition of exogenous galectin-1 to the cultures stimulated cell migration, suggesting that galectin-1 expression promotes tumor cell invasion and dissemination in vivo (83).

Camby et al also found that both low-grade and high-grade astrocytic tumors express galectin-1. However, while galectin-1 was expressed by both low-grade and high-grade tumors, the fraction of tumor area positive for galectin-1 by immunohistochemistry was greater in diffuse astrocytic tumors than low-grade tumors. Furthermore, high galectin-1 expression was associated with poor prognosis (13). To investigate the role of galectin-1 in tumor survival in vivo, human glioblastoma cells were grafted into brains of nude mice. Higher galectin-1 expression was observed in the invasive versus the non-invasive tumor areas. To directly examine the role of galectin-1 in glioblastoma cell growth and invasion, the human glioblastoma cells were stably transfected with galectin-1 antisense mRNA, to reduce galectin-1 expression, prior to grafting into nude mouse brain. Mice with xenografts expressing reduced levels of galectin-1 had an increased survival time compared with mice with xenografts expressing normal levels of galectin-1 (13).

To investigate possible mechanisms of galectin-1 induced astrocyte transformation, exogenous galectin-1 was added to the culture media of human neoplastic astrocytes. Galectin-1 caused increased cell motility associated with reorganization of the actin cytoskeleton and increased expression of RhoA, a protein that modulates actin polymerization and depolymerization. In concert with the data demonstrating that galectin-1 increases glioblastoma cell migration (83), these studies indicate that galectin-1 can promote detachment of tumor cells from the initial tumor site and facilitate migration into the surrounding brain parenchyma (13).

The influence of galectin-3 on brain malignancy is more controversial. Galectin-3 has recently been reported as a useful diagnostic marker to distinguish subtypes of astrocytic and glial tumors (66). Bresalier et al found a positive correlation between galectin-3 expression and tumor grade in diffuse astrocytomas in 71 patient samples, including 42 primary brain tumors and 29 metastases. In this study, no galectin-3 staining was observed in normal brain tissue, oligodendrogliomas, ependymomas, nor WHO grade 2 astrocytomas (sample size of 12) with the exception of occasional blood vessels, while 16 of 16 WHO grade 4 glioblastomas expressed galectin-3. Grade 3 anaplastic astrocytomas demonstrated intermediate galectin-3 expression with 3 of 4 samples staining weakly and 1 staining strongly. Galectin-3 localization in this study was primarily cytoplasmic, with occasional nuclear staining. Furthermore, all brain metastases investigated expressed galectin-3, with 9 of 10 patients having

higher galectin-3 expression in metastases than the primary tumors (8).

Kuklinski et al also found galectin-3 expression in 15 of 16 glioma cell lines tested, but not in normal astrocytes, oligodendrocytes, glial progenitor cells, or an oligodendrocyte precursor cell line. Galectin-3 was also expressed by one oligodendroglioma cell line, but not by a primitive neuroectodermal tumor or 4 glioblastoma cell lines. Galectin-3 localization in this study was primarily intracellular, although it was not clear whether the intracellular galectin-3 was cytoplasmic or nuclear. As mentioned above, recent work in prostate cancer has demonstrated opposing effects of cytosolic and nuclear galectin-3 on tumor cell growth and invasion, so precise cellular localization of galectins is critical for understanding galectin function in brain tumors. Galectin-3 may also have extracellular roles in brain cancer progression. As normal astrocytes, but not glioma cell lines, adhere to galectin-3, it has been proposed that defective tumor cell glycosylation may reduce available galectin-3 ligands on tumor cells; decreased glioma cell adhesion at the primary tumor site may contribute to glioma cell invasion and migration (48).

In contrast to the studies described above, Gordower et al found that white matter of non-cancerous brain expressed galectin-3. Furthermore, this group found a decrease in galectin-3 expression by tumor astrocytes during progression from low to high grade tumors, evidenced by a decrease in percent tissue area stained quantified by computer assisted microscopy, in an analysis of 84 astrocytic tumors, including 22 grade 2, 21 grade 3, 41 grade 4 astrocytomas, and 7 control samples. While this study found an overall inverse correlation between galectin-3 expression and tumor grade, the authors noted the emergence of tumor cell clones expressing high amounts of galectin-3 during the course of malignancy, suggesting that expression of galectin-3 in some subclones could promote tumor progression (25). Although this study did not specifically comment on galectin-3 localization within or outside the cell, the data presented in the paper indicated that immunoreactive galectin-3 was primarily cytoplasmic, with occasional staining of nuclei and ECM. The predominantly cytoplasmic localization of galectin-3 and the appearance of clones expressing high levels of galectin-3 suggest that, in these cells, intracellular galectin-3 may promote tumor growth by inhibiting apoptosis of tumor cells, as described above (25).

In a recent study by Debray et al, galectin-3 was shown to modulate human glioblastoma cell motility (19). In contrast to studies of extracellular galectin-1, which induced motility of human neoplastic astrocytes (13), human U373 glioblastoma cells expressing galectin-3 demonstrated diminished motility compared to U373 galectin-3 deficient cells created by expressing galectin-3 antisense mRNA (19). The galectin-3 deficient cells demonstrated increased expression of integrins, proteins known to be involved in regulation of cellular adhesion, suggesting a mechanism for the increased motility of galectin-3 deficient cells (19). The increase in cellular motility associated with diminished galectin-3 expression may in part explain the inverse correlation between galectin-3 and tumor grade observed in the studies by Gordower et al (25).

In addition to studies on expression of individual galectins, 2 studies have investigated the expression of multiple galectins in brain cancer. Lahm et al examined the expression of a panel of galectins, including galectin-1, -2, -3, -4, -7, -8, and -9, in 8 tumor cell lines. There was high expression of galectin-1, galectin-3, and galectin-8 by RT-PCR in all the cell lines, including those derived from neuroglioma, glioma, astrocytoma, and glioblastoma (49). Galectin-2 was expressed in one of the tumor cell lines, galectin-4 was detected inconsistently in 3 cell lines, low expression of galectin-9 was observed in 3 cell lines, while none of the samples expressed galectin-7. Thus, galectin-1, galectin-3 and galectin-8 appear to be the galectins most consistently expressed in brain cancer.

Expression of galectin-1, 3, and 8 were also examined in a series of 116 human astrocytic tumors, grades I-IV (12). Galectin-1 expression increased, while galectin-3 expression decreased, with increasing tumor grade. These 2 studies are concordant with the work described above demonstrating a consistent increase in galectin-1 expression with tumor grade, with conflicting observations on the relationship of galectin-3 and tumor grade. In xenografted glioblastomas, galectin-1, 3, and 8 were more highly expressed in the invasive sections of the tumors compared to non-invasive areas. Immobilized galectin-1 and galectin-3, and to a lesser extent galectin-8, stimulated in vitro glioblastoma cell migration, linking the extracellular expression of these galectins to cell motility, invasion and metastasis.

In contrast to the effects of galectin-1 and 3 in primary astrocytoma growth and progression, purified recombinant galectin-1 and 7, but not galectin-3, inhibited neuroblastoma cell growth in vitro. As extracellular galectin-1 and galectin-7 can both bind to the ganglioside GM1 on the surface of neuroblastoma cells, signaling through GM1 may participate in the growth inhibitory effects of galectin-1 and galectin-7 (45-47).

To understand the roles of galectins in neural tumor cell adhesion to the ECM or other cells, several groups have investigated the ability of neural cells and neural glycoproteins to bind to galectins. Dorsal root ganglia neurons adhere to a galectin-1 coated substratum, resulting in aggregation and formation of neurite bundles (67). Interactions between galectin-3 and neural tissue glycoproteins indicated a role for galectin-3 in neural cell-cell and cell-substrate adhesion (77). Similarly, neuroblastoma, pheochromocytoma, and transformed Schwann cell lines were found to adhere to galectin-3 (74). Furthermore, substrate-bound galectin-3 promoted neurite outgrowth from dorsal root ganglia explants, suggesting that galectin-3 interacts with glycoprotein or glycolipids ligands at the cell surface (74).

Any appropriately glycosylated glycoprotein or glycolipid may be a ligand for one or more galectins. Although many potential galectin ligands exist on neural cells, only 9 galectin-binding glycoproteins or glycolipids have been identified thus far. The HBGp82 glycoprotein was isolated from human brain as a ligand for galectin-1, while the glycolipid GM1 is a ligand for galectin-1 and galectin-7 on neuroblastoma cells. Several glycoproteins in brain have been identified as galectin-3 ligands, including N-CAM, L1, the myelin-associated glycoprotein MAG, and the ECM glycoproteins tenascin-C, tenascin-R, janusin, and restrictin (77). While relatively few galectin ligands have been identified in brain to date, many glycoproteins and glycolipids bear the appropriate carbohydrate epitopes that could be recognized by galectins; therefore, it is likely that many more galectin ligands will be identified in the future.

WHAT REMAINS TO BE DONE?

Future studies of galectins in primary brain cancer specimens need to incorporate precise information about subcellular localization of galectins in the tumor cells and the surrounding tissue. As discussed above, galectins can be localized in the cytosol or nucleus, associated with mitochondrial membranes, or targeted to the extracellular space, with the function of a particular galectin related to its localization. For example, while intracellular galectin-1 causes cell cycle arrest, extracellular galectin-1 induces proliferation or apoptosis.

Most tissues express multiple galectins, with different galectins having different biological functions, so that future studies must integrate information about the range of galectin family members expressed in tumors vs. normal tissues. Relating expression of one or two galectins to biologic outcome may not result in accurate conclusions, as additional galectins expressed in the tissues may directly regulate similar functions or influence the functions of other galectins (49, 50). These comprehensive studies will be critical for developing accurate algorithms that link expression patterns of specific galectins to prognosis of various types of brain cancer.

Analyses of galectin expression in brain cancer are beginning to incorporate studies of galectin function, eg, studies on effects of galectin-1 on astrocyte mobility have implicated increased expression of RhoA (13). However, it is essential that future studies continue to link galectin expression to specific functions.

Galectin function is not only dependent on localization within a cell, but also on the cell type involved; galectin-1 induces apoptosis of T-cells while promoting proliferation, migration, and invasiveness of glioblastoma cells (13, 23, 72). Few studies to date have taken an integrated approach to understanding the influence of different galectin functions on brain cancer progression. As many galectins are expressed in a wide range of neural and non-neural tissues, it will be critical to develop tissue-targeted approaches to regulate functions of specific galectins.

In addition, future work should continue to identify novel galectin ligands in normal and neoplastic cells, and to examine functional consequences associated with individual galectin-ligand interactions. As mentioned above, while few galectin ligands have been identified to date, many more glycoprotein and glycolipid ligands likely exist. Identifying galectin ligands may provide insight into signaling pathways triggered by these lectins in brain cancer.

In conclusion, galectins are a highly conserved, multifunctional family of proteins, with essential roles in tumorigenesis, cancer progression, and metastasis. The increasing availability of commercially available galectin reagents will facilitate integrative studies into precise subcellular localization, function, and signaling pathways of multiple galectins (Table 1). In addition, interested investigators will find a variety of resources, including microarrays and galectin-null mice, at the Consortium for Functional Glycomics, an NIH-funded program to encourage collaborative studies in lectin biology and to distribute resources to interested laboratories (*http://www.functionalglycomics.org*). The field will benefit from participation of translational and clinical investigators in order to maximize the potential of galectins as prognostic markers or as therapeutic targets for brain cancer.

gal-1	Antigenix America, Inc.	*http://www.antigenix.com*
	PeproTech	*http://www.peprotech.com*
	R&D Systems	*http://www.rndsystems.com*
gal-2	R&D Systems	*http://www.rndsystems.com*
gal-3	Abcam	*http://www.abcam.com*
	Affinity BioReagents	*http://www.bioreagents.com*
	BD Biosciences Pharmingen	*http://www.bdbiosciences.com*
	BioLegend	*http://www.biolegend.com*
	Chemicon	*http://www.chemicon.com*
	Lab Vision	*http://www.labvision.com*
	Novus Biologicals	*http://www.novus-biologicals.com*
	R&D Systems	*http://www.rndsystems.com*
gal-4	R&D Systems	*http://www.rndsystems.com*
gal-7	R&D Systems	*http://www.rndsystems.com*
gal-8	R&D Systems	*http://www.rndsystems.com*
gal-9	R&D Systems	*http://www.rndsystems.com*

Table 1. *Commercially available antibody reagents for human galectins.* The authors have not personally used all of these reagents and cannot attest to their reliability. Only antibodies to human gals are listed; antibodies to gals from other species are also available. Purified recombinant human gal-1, gal-2, gal-3, gal-4, gal-7, and gal-8 are available from R&D Systems.

ACKNOWLEDGMENTS

The authors would like to thank Drs Mike Teitell and Dennis Chute for critical reading of the manuscript. This work was supported by NIH grant R01 GM63281 to L.G.B.

REFERENCES

1. Ahmad N, Gabius HJ, Andre S, Kaltner H, Sabesan S, Roy R, Liu B, Macaluso F, Brewer CF (2004) Galectin-3 precipitates as a pentamer with synthetic multivalent carbohydrates and forms heterogeneous cross-linked complexes. *J Biol Chem* 279:10841-10847.

2. Ahmad N, Gabius HJ, Sabesan S, Oscarson S, Brewer CF (2004) Thermodynamic binding studies of bivalent oligosaccharides to galectin-1, galectin-3, and the carbohydrate recognition domain of galectin-3. *Glycobiology* 14:817-825.

3. Akahani S, Nangia-Makker P, Inohara H, Kim HR, Raz A (1997) Galectin-3: a novel antiapoptotic molecule with a functional BH1 (NWGR) domain of Bcl-2 family. *Cancer Res* 57:5272-5276.

4. Allen HJ, Sharma A, Ahmed H, Piver MS, Gamarra M (1993) Galaptin and galaptin-binding glycoconjugates in serum and effusions of carcinoma patients. *Tumour Biol* 14:360-368.

5. Allione A, Wells V, Forni G, Mallucci L, Novelli F (1998) Beta-galactoside-binding protein (beta GBP) alters the cell cycle, up-regulates expression of the alpha- and beta-chains of the IFN-gamma receptor, and triggers IFN-gamma-mediated apoptosis of activated human T lymphocytes. *J Immunol* 161:2114-2119.

6. Bernerd F, Sarasin A, Magnaldo T (1999) Galectin-7 overexpression is associated with the apoptotic process in UVB-induced sunburn keratinocytes. *Proc Natl Acad Sci U S A* 96:11329-11334.

7. Bladier D, Joubert R, Avellana-Adalid V, Kemeny JL, Doinel C, Amouroux J, Caron M (1989) Purification and characterization of a galactoside-binding lectin from human brain. *Arch Biochem Biophys* 269:433-439.

8. Bresalier RS, Yan PS, Byrd JC, Lotan R, Raz A (1997) Expression of the endogenous galactose-binding protein galectin-3 correlates with the

malignant potential of tumors in the central nervous system. *Cancer* 80:776-787.

9. Brewer CF (2004) Thermodynamic binding studies of galectin-1, -3 and -7. *Glycoconj J* 19:459-465.

10. Brunger AT, Milburn MV, Tong L, deVos AM, Jancarik J, Yamaizumi Z, Nishimura S, Ohtsuka E, Kim SH (1990) Crystal structure of an active form of RAS protein, a complex of a GTP analog and the HRAS p21 catalytic domain. *Proc Natl Acad Sci U S A* 87:4849-4853.

11. Califice S, Castronovo V, Bracke M, Van Den Brule F (2004) Dual activities of galectin-3 in human prostate cancer: tumor suppression of nuclear galectin-3 vs tumor promotion of cytoplasmic galectin-3. *Oncogene* 23:7527-7523.

12. Camby I, Belot N, Rorive S, Lefranc F, Maurage CA, Lahm H, Kaltner H, Hadari Y, Ruchoux MM, Brotchi J, Zick Y, Salmon I, Gabius HJ, Kiss R (2001) Galectins are differentially expressed in supratentorial pilocytic astrocytomas, astrocytomas, anaplastic astrocytomas and glioblastomas, and significantly modulate tumor astrocyte migration. *Brain Pathol* 11:12-26.

13. Camby I, Belot N, Lefranc F, Sadeghi N, de Launoit Y, Kaltner H, Musette S, Darro F, Danguy A, Salmon I, Gabius HJ, Kiss R (2002) Galectin-1 modulates human glioblastoma cell migration into the brain through modifications to the actin cytoskeleton and levels of expression of small GTPases. *J Neuropathol Exp Neurol* 61:585-596.

14. Caron M, Joubert R, Bladier D (1987) Purification and characterization of a beta-galactoside-binding soluble lectin from rat and bovine brain. *Biochim Biophys Acta* 925:290-296.

15. Casey MB, Lohse CM, Lloyd RV (2003) Distinction between papillary thyroid hyperplasia and papillary thyroid carcinoma by immunohistochemical staining for cytokeratin 19, galectin-3, and HBME-1. *Endocr Pathol* 14:55-60.

16. Cooper DN, Barondes SH (1990) Evidence for export of a muscle lectin from cytosol to extracellular matrix and for a novel secretory mechanism. *J Cell Biol* 110:1681-1691.

17. Cooper DN (2002) Galectinomics: finding themes in complexity. *Biochim Biophys Acta* 1572:209-231.

18. Cornillot JD, Pontet M, Dupuy C, Chadli A, Caron M, Joubert-Caron R, Bourin P, Bladier D (1998) Production and characterization of a monoclonal antibody able to discriminate galectin-1 from galectin-2 and galectin-3. *Glycobiology* 8:425-432.

19. Debray C, Vereecken P, Belot N, Teillard P, Brion JP, Pandolfo M, Pochet R (2004) Multifaceted role of galectin-3 on human glioblastoma cell motility. *Biochem Biophys Res Commun* 325:1393-1398.

20. Ellerhorst J, Nguyen T, Cooper DN, Estrov Y, Lotan D, Lotan R (1999) Induction of differentiation and apoptosis in the prostate cancer cell line LNCaP by sodium butyrate and galectin-1. *Int J Oncol* 14:225-232.

21. Finley DJ, Arora N, Zhu B, Gallagher L, Fahey TJ, 3rd (2004) Molecular profiling distinguishes papillary carcinoma from benign thyroid nodules. *J Clin Endocrinol Metab* 89:3214-3223.

22. Fukumori T, Takenaka Y, Yoshii T, Kim HR, Hogan V, Inohara H, Kagawa S, Raz A (2003) CD29 and CD7 mediate galectin-3-induced type II T-cell apoptosis. *Cancer Res* 63:8302-8311.

23. Gabius HJ, Andre S, Gunsenhauser I, Kaltner H, Kayser G, Kopitz J, Lahm H, Harms D, Szymas J, Kayser K (2002) Association of galectin-1- but not galectin-3-dependent parameters with proliferation activity in human neuroblastomas and small cell lung carcinomas. *Anticancer Res* 22:405-410.

24. Gillenwater A, Xu XC, el-Naggar AK, Clayman GL, Lotan R (1996) Expression of galectins in head and neck squamous cell carcinoma. *Head Neck* 18:422-432.

25. Gordower L, Decaestecker C, Kacem Y, Lemmers A, Gusman J, Burchert M, Danguy A, Gabius H, Salmon I, Kiss R, Camby I (1999) Galectin-3 and galectin-3-binding site expression in human adult astrocytic tumours and related angiogenesis. *Neuropathol Appl Neurobiol* 25:319-330.

26. Gray CA, Adelson DL, Bazer FW, Burghardt RC, Meeusen EN, Spencer TE (2004) Discovery and characterization of an epithelial-specific galectin in the endometrium that forms crystals in the trophectoderm. *Proc Natl Acad Sci U S A* 101:7982-7987.

27. Gu M, Wang W, Song WK, Cooper DN, Kaufman SJ (1994) Selective modulation of the interaction of alpha 7 beta 1 integrin with fibronectin and laminin by L-14 lectin during skeletal muscle differentiation. *J Cell Sci* 107 (Pt 1):175-181.

28. Hadari YR, Arbel-Goren R, Levy Y, Amsterdam A, Alon R, Zakut R, Zick Y (2000) Galectin-8 binding to integrins inhibits cell adhesion and induces apoptosis. *J Cell Sci* 113 (Pt 13):2385-2397.

29. Hamann KK, Cowles EA, Wang JL, Anderson RL (1991) Expression of carbohydrate binding protein 35 in human fibroblasts: variations in the levels of mRNA, protein, and isoelectric species as a function of replicative competence. *Exp Cell Res* 196:82-91.

30. He J, Baum LG (2004) Presentation of galectin-1 by extracellular matrix triggers T-cell death. *J Biol Chem* 279:4705-4712.

31. Hernandez JD, Baum LG (2002) Ah, sweet mystery of death! Galectins and control of cell fate. *Glycobiology* 12:127R-136R.

32. Holland EC (2001) Brain tumor animal models: importance and progress. *Curr Opin Oncol* 13:143-147.

33. Honjo Y, Nangia-Makker P, Inohara H, Raz A (2001) Down-regulation of galectin-3 suppresses tumorigenicity of human breast carcinoma cells. *Clin Cancer Res* 7:661-668.

34. Horie H, Kadoya T, Hikawa N, Sango K, Inoue H, Takeshita K, Asawa R, Hiroi T, Sato M, Yoshioka T, Ishikawa Y (2004) Oxidized galectin-1 stimulates macrophages to promote axonal regeneration in peripheral nerves after axotomy. *J Neurosci* 24:1873-1880.

35. Hoyer KK, Pang M, Gui D, Shintaku IP, Kuwabara I, Liu FT, Said JW, Baum LG, Teitell MA (2004) An anti-apoptotic role for galectin-3 in diffuse large B-cell lymphomas. *Am J Pathol* 164:893-902.

36. Hughes RC (2001) Galectins as modulators of cell adhesion. *Biochimie* 83:667-676.

37. Hynes MA, Gitt M, Barondes SH, Jessell TM, Buck LB (1990) Selective expression of an endogenous lactose-binding lectin gene in subsets of central and peripheral neurons. *J Neurosci* 10:1004-1013.

38. Inohara H, Honjo Y, Yoshii T, Akahani S, Yoshida J, Hattori K, Okamoto S, Sawada T, Raz A, Kubo T (1999) Expression of galectin-3 in fine-needle aspirates as a diagnostic marker differentiating benign from malignant thyroid neoplasms. *Cancer* 85:2475-2484.

39. Iurisci I, Tinari N, Natoli C, Angelucci D, Cianchetti E, Iacobelli S (2000) Concentrations of galectin-3 in the sera of normal controls and cancer patients. *Clin Cancer Res* 6:1389-1393.

40. Joo HG, Goedegebuure PS, Sadanaga N, Nagoshi M, von Bernstorff W, Eberlein TJ (2001) Expression and function of galectin-3, a beta-galactoside-binding protein in activated T lymphocytes. *J Leukoc Biol* 69:555-564.

41. Joubert R, Kuchler S, Zanetta JP, Bladier D, Avellana-Adalid V, Caron M, Doinel C, Vincendon G (1989) Immunohistochemical localization of a beta-galactoside-binding lectin in rat central nervous system. I. Light- and electron-microscopical studies on developing cerebral cortex and corpus callosum. *Dev Neurosci* 11:397-413.

42. Kageshita T, Kashio Y, Yamauchi A, Seki M, Abedin MJ, Nishi N, Shoji H, Nakamura T, Ono T, Hirashima M (2002) Possible role of galectin-9 in cell aggregation and apoptosis of human melanoma cell lines and its clinical significance. *Int J Cancer* 99:809-816.

43. Kim HR, Lin HM, Biliran H, Raz A (1999) Cell cycle arrest and inhibition of anoikis by galectin-3 in human breast epithelial cells. *Cancer Res* 59:4148-4154.

44. Konstantinov KN, Robbins BA, Liu FT (1996) Galectin-3, a beta-galactoside-binding animal lectin, is a marker of anaplastic large-cell lymphoma. *Am J Pathol* 148:25-30.

45. Kopitz J, von Reitzenstein C, Burchert M, Cantz M, Gabius HJ (1998) Galectin-1 is a major receptor for ganglioside GM1, a product of the growth-controlling activity of a cell surface ganglioside sialidase, on human neuroblastoma cells in culture. *J Biol Chem* 273:11205-11211.

46. Kopitz J, von Reitzenstein C, Andre S, Kaltner H, Uhl J, Ehemann V, Cantz M, Gabius HJ (2001) Negative regulation of neuroblastoma cell growth by carbohydrate-dependent surface binding of galectin-1 and functional divergence from galectin-3. *J Biol Chem* 276:35917-35923.

47. Kopitz J, Andre S, von Reitzenstein C, Versluis K, Kaltner H, Pieters RJ, Wasano K, Kuwabara I, Liu FT, Cantz M, Heck AJ, Gabius HJ (2003) Homodimeric galectin-7 (p53-induced gene 1) is a negative growth regulator for human neuroblastoma cells. *Oncogene* 22:6277-6288.

48. Kuklinski S, Pesheva P, Heimann C, Urschel S, Gloor S, Graeber S, Herzog V, Pietsch T, Wiestler

OD, Probstmeier R (2000) Expression pattern of galectin-3 in neural tumor cell lines. *J Neurosci Res* 60:45-57.

49. Lahm H, Andre S, Hoeflich A, Fischer JR, Sordat B, Kaltner H, Wolf E, Gabius HJ (2001) Comprehensive galectin fingerprinting in a panel of 61 human tumor cell lines by RT-PCR and its implications for diagnostic and therapeutic procedures. *J Cancer Res Clin Oncol* 127:375-386.

50. Lahm H, Andre S, Hoeflich A, Kaltner H, Siebert HC, Sordat B, von der Lieth CW, Wolf E, Gabius HJ (2004) Tumor galectinology: insights into the complex network of a family of endogenous lectins. *Glycoconj* J 20:227-238.

51. Le Marer N, Hughes RC (1996) Effects of the carbohydrate-binding protein galectin-3 on the invasiveness of human breast carcinoma cells. *J Cell Physiol* 168:51-58.

52. Lee YJ, Song YK, Song JJ, Siervo-Sassi RR, Kim HR, Li L, Spitz DR, Lokshin A, Kim JH (2003) Reconstitution of galectin-3 alters glutathione content and potentiates TRAIL-induced cytotoxicity by dephosphorylation of Akt. *Exp Cell Res* 288:21-34.

53. Lipkowitz MS, Leal-Pinto E, Cohen BE, Abramson RG (2004) Galectin 9 is the sugar-regulated urate transporter/channel UAT. *Glycoconj J* 19:491-498.

54. Lopez-Lucendo MF, Solis D, Andre S, Hirabayashi J, Kasai K, Kaltner H, Gabius HJ, Romero A (2004) Growth-regulatory human galectin-1: crystallographic characterisation of the structural changes induced by single-site mutations and their impact on the thermodynamics of ligand binding. *J Mol Biol* 343:957-970.

55. Lotan R, Belloni PN, Tressler RJ, Lotan D, Xu XC, Nicolson GL (1994) Expression of galectins on microvessel endothelial cells and their involvement in tumour cell adhesion. *Glycoconj J* 11:462-468.

56. Louis DN, Holland EC, Cairncross JG (2001) Glioma classification: a molecular reappraisal. *Am J Pathol* 159:779-786.

57. Matarrese P, Fusco O, Tinari N, Natoli C, Liu FT, Semeraro ML, Malorni W, Iacobelli S (2000) Galectin-3 overexpression protects from apoptosis by improving cell adhesion properties. *Int J Cancer* 85:545-554.

58. McGraw J, McPhail LT, Oschipok LW, Horie H, Poirier F, Steeves JD, Ramer MS, Tetzlaff W (2004) Galectin-1 in regenerating motoneurons. *Eur J Neurosci* 20:2872-2880.

59. McGraw J, Oschipok LW, Liu J, Hiebert GW, Mak CF, Horie H, Kadoya T, Steeves JD, Ramer MS, Tetzlaff W (2004) Galectin-1 expression correlates with the regenerative potential of rubrospinal and spinal motoneurons. *Neuroscience* 128:713-719.

60. Mischel PS, Cloughesy TF, Nelson SF (2004) DNA-microarray analysis of brain cancer: molecular classification for therapy. *Nat Rev Neurosci* 5:782-792.

61. Moisan S, Demers M, Mercier J, Magnaldo T, Potworowski EF, St-Pierre Y (2003) Upregulation of galectin-7 in murine lymphoma cells is associated with progression toward an aggressive phenotype. *Leukemia* 17:751-759.

62. Moiseeva EP, Spring EL, Baron JH, de Bono DP (1999) Galectin 1 modulates attachment, spreading and migration of cultured vascular smooth muscle cells via interactions with cellular receptors and components of extracellular matrix. *J Vasc Res* 36:47-58.

63. Moon BK, Lee YJ, Battle P, Jessup JM, Raz A, Kim HR (2001) Galectin-3 protects human breast carcinoma cells against nitric oxide-induced apoptosis: implication of galectin-3 function during metastasis. *Am J Pathol* 159:1055-1060.

64. Moutsatsos IK, Wade M, Schindler M, Wang JL (1987) Endogenous lectins from cultured cells: nuclear localization of carbohydrate-binding protein 35 in proliferating 3T3 fibroblasts. *Proc Natl Acad Sci U S A* 84:6452-6456.

65. Nakamura M, Inufusa H, Adachi T, Aga M, Kurimoto M, Nakatani Y, Wakano T, Nakajima A, Hida JI, Miyake M, Shindo K, Yasutomi M (1999) Involvement of galectin-3 expression in colorectal cancer progression and metastasis. *Int J Oncol* 15:143-148.

66. Neder L MS, Carlotti CG Jr, Gabbai AA, Rosemberg S, Malheiros SM, Siqueira RP O-SS, Uno M, Aguiar PH, Miura F, Chammas R, Colli BO, Silva WA Jr ZM (2004) Galectin-3 as an immunohistochemical tool to distinguish pilocytic astrocytomas from diffuse astrocytomas, and glioblastomas from anaplastic oligodendrogliomas. *Brain Pathol* 14:399-405.

67. Outenreath RL, Jones AL (1992) Influence of an endogenous lectin substrate on cultured dorsal root ganglion cells. *J Neurocytol* 21:788-795.

68. Park JW, Voss PG, Grabski S, Wang JL, Patterson RJ (2001) Association of galectin-1 and galectin-3 with Gemin4 in complexes containing the SMN protein. *Nucleic Acids Res* 29:3595-3602.

69. Paron I, Scaloni A, Pines A, Bachi A, Liu F-T, Puppin C, Pandolfi M, Ledda L, Di Loreto C, Damante G, Tell G (2003) Nuclear localization of Galectin-3 in transformed thyroid cells: a role in transcriptional regulation. *Biochem Biophys Res Commun* 302:545-553.

70. Paz A, Haklai R, Elad-Sfadia G, Ballan E, Kloog Y (2001) Galectin-1 binds oncogenic H-Ras to mediate Ras membrane anchorage and cell transformation. *Oncogene* 20:7486-7493.

71. Pellizzoni L, Kataoka N, Charroux B, Dreyfuss G (1998) A novel function for SMN, the spinal muscular atrophy disease gene product, in pre-mRNA splicing. *Cell* 95:615-624.

72. Perillo NL, Pace KE, Seilhamer JJ, Baum LG (1995) Apoptosis of T-cells mediated by galectin-1. *Nature* 378:736-739.

73. Perillo NL, Uittenbogaart CH, Nguyen JT, Baum LG (1997) Galectin-1, an endogenous lectin produced by thymic epithelial cells, induces apoptosis of human thymocytes. *J Exp Med* 185:1851-1858.

74. Pesheva P, Kuklinski S, Schmitz B, Probstmeier R (1998) Galectin-3 promotes neural cell adhesion and neurite growth. *J Neurosci Res* 54:639-654.

75. Pesheva P, Urschel S, Frei K, Probstmeier R (1998) Murine microglial cells express functionally active galectin-3 in vitro. *J Neurosci Res* 51:49-57.

76. Pesheva P, Kuklinski S, Biersack HJ, Probstmeier R (2000) Nerve growth factor-mediated expression of galectin-3 in mouse dorsal root ganglion neurons. *Neurosci Lett* 293:37-40.

77. Probstmeier R, Montag D, Schachner M (1995) Galectin-3, a beta-galactoside-binding animal lectin, binds to neural recognition molecules. *J Neurochem* 64:2465-2472.

78. Puche AC, Poirier F, Hair M, Bartlett PF, Key B (1996) Role of galectin-1 in the developing mouse olfactory system. *Dev Biol* 179:274-287.

79. Rabinovich GA, Baum LG, Tinari N, Paganelli R, Natoli C, Liu FT, Iacobelli S (2002) Galectins and their ligands: amplifiers, silencers or tuners of the inflammatory response? *Trends Immunol* 23:313-320.

80. Rabinovich GA, Rubinstein N, Matar P, Rozados V, Gervasoni S, Scharovsky GO (2002) The antimetastatic effect of a single low dose of cyclophosphamide involves modulation of galectin-1 and Bcl-2 expression. *Cancer Immunol Immunother* 50:597-603.

81. Regan LJ, Dodd J, Barondes SH, Jessell TM (1986) Selective expression of endogenous lactose-binding lectins and lactoseries glycoconjugates in subsets of rat sensory neurons. *Proc Natl Acad Sci U S A* 83:2248-2252.

82. Reichert F, Saada A, Rotshenker S (1994) Peripheral nerve injury induces Schwann cells to express two macrophage phenotypes: phagocytosis and the galactose-specific lectin MAC-2. *J Neurosci* 14:3231-3245.

83. Rorive S, Belot N, Decaestecker C, Lefranc F, Gordower L, Micik S, Maurage CA, Kaltner H, Ruchoux MM, Danguy A, Gabius HJ, Salmon I, Kiss R, Camby I (2001) Galectin-1 is highly expressed in human gliomas with relevance for modulation of invasion of tumor astrocytes into the brain parenchyma. *Glia* 33:241-255.

84. Rossi DL, Acebron A, Santisteban P (1995) Function of the homeo and paired domain proteins TTF-1 and Pax-8 in thyroid cell proliferation. *J Biol Chem* 270:23139-23142.

85. Rubinstein N, Alvarez M, Zwirner NW, Toscano MA, Ilarregui JM, Bravo A, Mordoh J, Fainboim L, Podhajcer OL, Rabinovich GA (2004) Targeted inhibition of galectin-1 gene expression in tumor cells results in heightened T-cell-mediated rejection; A potential mechanism of tumor-immune privilege. *Cancer Cell* 5:241-251.

86. Saggiorato E, Cappia S, De Giuli P, Mussa A, Pancani G, Caraci P, Angeli A, Orlandi F (2001) Galectin-3 as a presurgical immunocytodiagnostic marker of minimally invasive follicular thyroid carcinoma. *J Clin Endocrinol Metab* 86:5152-5158.

87. Sango K, Tokashiki A, Ajiki K, Horie M, Kawano H, Watabe K, Horie H, Kadoya T (2004) Synthesis, localization and externalization of galectin-1 in mature dorsal root ganglion neurons and Schwann cells. *Eur J Neurosci* 19:55-64.

88. Sanjuan X, Fernandez PL, Castells A, Castronovo V, van den Brule F, Liu FT, Cardesa A, Campo E (1997) Differential expression of galectin 3 and galectin 1 in colorectal cancer progression. *Gastroenterology* 113:1906-1915.

89. Schoeppner HL, Raz A, Ho SB, Bresalier RS (1995) Expression of an endogenous galactose-binding lectin correlates with neoplastic progression in the colon. *Cancer* 75:2818-2826.

90. Skrincosky DM, Allen HJ, Bernacki RJ (1993) Galaptin-mediated adhesion of human ovarian carcinoma A121 cells and detection of cellular galaptin-binding glycoproteins. *Cancer Res* 53:2667-2675.

91. Stierstorfer B, Kaltner H, Neumuller C, Sinowatz F, Gabius HJ (2000) Temporal and spatial regulation of expression of two galectins during kidney development of the chicken. *Histochem J* 32:325-336.

92. Sturm A, Lensch M, Andre S, Kaltner H, Wiedenmann B, Rosewicz S, Dignass AU, Gabius HJ (2004) Human galectin-2: novel inducer of T-cell apoptosis with distinct profile of caspase activation. *J Immunol* 173:3825-3837.

93. van den Brule F, Califice S, Castronovo V (2004) Expression of galectins in cancer: a critical review. *Glycoconj J* 19:537-542.

94. van den Brule FA, Buicu C, Baldet M, Sobel ME, Cooper DN, Marschal P, Castronovo V (1995) Galectin-1 modulates human melanoma cell adhesion to laminin. *Biochem Biophys Res Commun* 209:760-767.

95. van den Brule FA, Waltregny D, Castronovo V (2001) Increased expression of galectin-1 in carcinoma-associated stroma predicts poor outcome in prostate carcinoma patients. *J Pathol* 193:80-87.

96. Vespa GN, Lewis LA, Kozak KR, Moran M, Nguyen JT, Baum LG, Miceli MC (1999) Galectin-1 specifically modulates TCR signals to enhance TCR apoptosis but inhibit IL-2 production and proliferation. *J Immunol* 162:799-806.

97. Visegrady B, Than NG, Kilar F, Sumegi B, Than GN, Bohn H (2001) Homology modelling and molecular dynamics studies of human placental tissue protein 13 (galectin-13). *Protein Eng* 14:875-880.

98. Wada J, Ota K, Kumar A, Wallner EI, Kanwar YS (1997) Developmental regulation, expression, and apoptotic potential of galectin-9, a beta-galactoside binding lectin. *J Clin Invest* 99:2452-2461.

99. Warfield PR, Makker PN, Raz A, Ochieng J (1997) Adhesion of human breast carcinoma to extracellular matrix proteins is modulated by galectin-3. *Invasion Metastasis* 17:101-112.

100. Wechsler-Reya R, Scott MP (2001) The developmental biology of brain tumors. *Annu Rev Neurosci* 24:385-428.

101. Wells V, Davies D, Mallucci L (1999) Cell cycle arrest and induction of apoptosis by beta galactoside binding protein (beta GBP) in human mammary cancer cells. A potential new approach to cancer control. *Eur J Cancer* 35:978-983.

102. Woo HJ, Shaw LM, Messier JM, Mercurio AM (1990) The major non-integrin laminin binding protein of macrophages is identical to carbohydrate binding protein 35 (Mac-2). *J Biol Chem* 265:7097-7099.

103. Yamaoka K, Mishima K, Nagashima Y, Asai A, Sanai Y, Kirino T (2000) Expression of galectin-1 mRNA correlates with the malignant potential of human gliomas and expression of antisense galectin-1 inhibits the growth of 9 glioma cells. *J Neurosci Res* 59:722-730.

104. Yang RY, Hsu DK, Liu FT (1996) Expression of galectin-3 modulates T-cell growth and apoptosis. *Proc Natl Acad Sci U S A* 93:6737-6742.

105. Yang RY, Hsu DK, Yu L, Ni J, Liu FT (2001) Cell cycle regulation by galectin-12, a new member of the galectin superfamily. *J Biol Chem* 276:20252-20260.

106. Yoshii T, Inohara H, Takenaka Y, Honjo Y, Akahani S, Nomura T, Raz A, Kubo T (2001) Galectin-3 maintains the transformed phenotype of thyroid papillary carcinoma cells. *Int J Oncol* 18:787-792.

107. Zhou Q, Cummings RD (1993) L-14 lectin recognition of laminin and its promotion of in vitro cell adhesion. *Arch Biochem Biophys* 300:6-17.