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Regulation of cellular homeostasis by galectins

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Members of the galectin family are presently known to participate in cellular homeostasis by modulating cell growth, controlling cell cycle progression, and inducing or inhibiting apoptosis. Both intracellular and extracellular activities of galectins have been described, with the former typically independent of lectin activity, and the latter mediated by lectin activity. Galectin-1 and -3 are recognized as activators and inducers of cell stasis in extracellular capacities. Galectin-1, -7, -8, -9 and -12 are characterized as promoters or inducers of apoptosis, while galectin-3 is demonstrated as an inhibitor of apoptosis intracellularly. Localization studies of galectins have established that these proteins can segregate into multiple intracellular compartments, and the preference for segregation is dependent on the status of the cell. Localization would, therefore, likely correspond to compartmental function. While galectin-1 and -3 have been the most abundantly expressed and extensively studied, and therefore, the members best understood, expanding interest in galectins has resulted in description of new members that display more restricted expression patterns, suggesting more specific activity. Nevertheless, as demonstrated for many members, it appears that a major feature of the galectin family is the homeostatic regulation of cells.
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Abbreviations: **CRD, carbohydrate-binding domain; IFNγ, interferon γ; LPS, lipopolysaccharide; TNFα, tumor necrosis factor α; UVB, ultraviolet B light.**

Introduction

Among the moderately conserved group of proteins present in a mammalian cell is an ancient family of proteins called galectins. These proteins have been identified in phylogenically distant organisms and are observed in vertebrates, insects, nematodes and fungi. Some galectins demonstrate polymorphisms and are presently most apparent in mammals. Galectin homologs are also present in plants (phylogenic distribution compiled in the SMART database, http://smart.embl-heidelberg.de/) [1], suggesting primeval significance, though they are curiously absent in yeast (*Saccharomyces*). All members of this family are composed of a distinct carbohydrate-binding domain (CRD) with conserved consensus regions and galactose-specific lectin activity. Thus far, members of this family contain a single CRD domain (galectin-1, -2, -5, -7, -10, -11, -13 and -14), two domains interconnected by an unconserved linker region (galectin-4, -6, -8, -9 and -12) or a chimeric structure consisting of a single CRD domain and an extended N-terminal region as represented by galectin-3. Homologues with little or no lectin activity have also been identified, such as the adhesive protein from the lens of eye, galectin related interfiber protein (GRIFIN) [2].

Galectins have demonstrated extracellular functions as one might expect from the abundance of glycoproteins oriented extracellularly, but many are functional intracellularly in keeping with predominant localization within cells. While these proteins may participate in fundamental tasks associated with lectin activity, they have been adapted to interact with other proteins in a lectin-independent manner, thus expanding the sphere of influence of galectins.

Extracellular functions of galectins were revealed by studies of lectin introduced exogenously, while many intracellular properties of galectins were elucidated by extrinsic control of gene expression in cells via transfection and inhibition (*e.g.*, antisense). Current technologies permitting manipulation of gene expression in experimental animals have confirmed some functions of galectin-1 and -3, and uncovered roles of these lectins in tissues not previously studied. What has been observed with respect to these two is that secretion can result in extracellular functions such as cell activation via autocrine or paracrine mechanisms, and direct mediation of homotypic cell interaction or adhesion to the extracellular matrix. In the meantime, being present in the cytoplasm and nucleus, they exert intracellular functions as exemplified by

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regulation of cell proliferation and apoptosis, and RNA processing. This pattern of distribution and regulation of cell proliferation and survival appears to be a hallmark of the galectin family.

The presence of more than a dozen members within the galectin family and the pronounced phylogenic conservation of galectins from sponges to mammals are suggestive of their importance within the organism. Recent studies reveal that these proteins are often multifunctional and multi-compartmented in cells in a regulated manner. This review describes the role of galectins in cellular homeostasis, focusing on the regulation of cell growth, apoptosis and the cell cycle.

Galectin-1

Regulation of cell growth

Galectin-1 has been shown to either promote or inhibit cell growth in different cell types when added exogenously to cells, as described below. It is mitogenic for vascular endothelial cells; the activity is related to its lectin properties and relatively high concentrations are required (3.4 μM), although activity was observed at 0.26 μM under low serum conditions [3]. It also promotes the growth of 3T3 fibroblasts [4]. Galectin-1 has been observed to enhance axonal regeneration at very low concentrations of 3.4 pM, but this function is unrelated to its lectin properties [5].

Inhibition of cell growth by galectin-1 has been documented for neuroblastoma cells, and this occurs through its carbohydrate-binding properties [6]. However, it also inhibits the growth of other cell types in a manner independent of its lectin properties, including mouse embryonic fibroblasts [7], concanavalin A (Con A)-stimulated rat spleen mononuclear cells [8], phytohemagglutinin (PHA)-activated T lymphocytes and T lymphoma cells [9], and naive and antigen-experienced CD8+ T cells [10]. These effects were observed at concentrations of 2.7–94 nM.

The growth regulatory activity of galectin-1 has also been shown through modulating its gene expression. Inhibition of galectin-1 expression in a rat glioma cell line by transfecting the cells with antisense galectin-1 cDNA arrests the cell growth [11], suggesting that endogenous galectin-1 has a growth-promoting role.

Therefore, the effects of galectin-1 appear to be multifaceted. It can function in both carbohydrate-dependent and carbohydrate-independent fashion and its effects can be either positive or negative, depending on the responder cell types. The carbohydrate-dependent activities are typically demonstrated with relatively high concentrations, while the carbohydrate-independent activities can be shown with substantially lower concentrations. Since galectin-1 is known to induce cell death (see below), it is possible that whether it is mitogenic or inhibitory depends on whether it causes cell death in the particular cell type being studied. If it does not cause cell death, then the outcome is more likely to be cell proliferation. However, this analysis is not supported by a report showing the biphasic modulation of cell growth by recombinant galectin-1. While high doses of galectin-1 inhibit cell proliferation independent of its sugar-binding activity, low doses of galectin-1 are mitogenic and are susceptible to inhibition by lactose [12].

The mechanism of galectin-1-induced cell growth promotion or inhibition remains to be elucidated. In the case of the response that is dependent on its lectin properties, the effect is likely due to the engagement of cell surface glycoconjugates. In this regard, ganglioside GM1 has been suggested as a ligand of galectin-1 and a possible candidate cell surface molecule mediating galectin-1's growth-inhibitory effect on neuroblastoma cells [6].

Regulation of apoptosis

Galectin-1 added extracellularly has been shown to induce apoptosis in activated human T cells, human T leukemia cell lines, and subsets of CD4(lo) CD8(lo) thymocytes [13–16]. Relatively high concentrations ($\sim$10 μM) are required and the effect is dependent on N-glycans expressed on the cell surfaces. Galectin-1 has also been shown to induce apoptosis in human mammary cancer cells [17] and prostate cancer cell line LNCaP [18].

Galectin-1 binds to a restricted set of T cell surface glycoproteins, including CD45, CD43, and CD7 [19]. This binding results in a redistribution of these glycoproteins into segregated membrane microdomains. While CD45 and CD3 colocalize on large islands of apoptotic blebs, CD7 and CD43 colocalize in small patches away from apoptotic blebs [19]. The possible involvement of CD45 in galectin-1-induced apoptosis has been demonstrated in a number of investigations [20,21], but appears to be controversial [22].

Regarding the mechanisms of galectin-1-induced apoptosis, it has been shown that galectin-1 induces up-regulation of the expression of both the α- and β-chains of the interferon-gamma (IFN-γ) receptor on activated T lymphocytes, rendering the cells sensitive to IFN-γ-induced apoptosis [9]. It has also been shown that galectin-1 causes a decrease of the anti-apoptotic protein Bcl-2, and thus predominance of the pro-apoptotic protein Bax [23], and enhances extracellular signal-regulated kinase-2 (ERK-2) activation [16].

In other studies, galectin-1 has demonstrated inhibitory effects on cell adhesion through interactions with laminin, $\alpha_7\beta_1$ integrin, fibronectin, and collagen type IV [24–26]. Though no consequences in cell survival were reported in these studies, loss of cell adhesion may result in apoptosis.

Regulation of the cell cycle

Extracellular galectin-1 has been shown to induce cell cycle arrest during the S to G2 transition of mammary cell lines [27], and has also been shown to arrest T lymphocytes in the S and

G2/M phases of the cell cycle [23]. The mechanisms underlying these effects remain to be determined.

Galectin-1 may also interfere with normal cell cycle regulation by contributing to cell transformation via direct interaction with oncogenic Ras. This interaction is dependent on activation of, and specific for membrane localization of oncogenic-Ras but not wild type Ras and contributes to enhanced Ras signaling [28]. Further studies demonstrated that galectin-1 imparts selectivity to Ras signaling toward Raf over the phospshonositide 3-kinase pathway [29], and that this lectin promotes partitioning of activated H-Ras into membrane nonraft microdomains [30].

Galectin-3

Regulation of cell growth

The role of galectin-3 in regulation of cell growth has been demonstrated with various cell types by different approaches. Extracellular galectin-3 has been shown to stimulate growth of fibroblasts [31] and mesangial cells [32] and promote outgrowth of neurites from dorsal root ganglia explants [33]. Galectin-3 also stimulates capillary tube formation of human umbilical vein endothelial cell *in vitro* and angiogenesis *in vivo* [34]. These effects are dependent on its lectin properties and effective concentrations are submicromolar to micromolar. Galectin-3 can also be a negative growth regulator. Exogenously added galectin-3 was shown to inhibit the growth of Madin-Darby canine kidney (MDCK), but not mutant cells that are defective in glycan synthesis and thus lacking cell surface glycoligands for galectin-3 [35]. Galectin-3 also inhibits proliferation of bone marrow cells induced by recombinant granulocyte-macrophage colony-stimulating factor (GM-CSF) [36].

Transfectants of the human T lymphoma Jurkat cells ectopically expressing galectin-3 were found to grow more efficiently than control transfectants not expressing this lectin, especially under suboptimal growth conditions (*e.g.*, in culture medium containing only 1% fetal bovine serum) [37]. Evidence for a role of endogenous galectin-3 in regulation of cell growth has also been provided by a number of studies with different cell lines, in which galectin-3 expression is suppressed by antisense oligonucleotides or antisense cDNA. These include the growth of human breast cancer cells [38,39], proliferation of T lymphocytes induced by mitogen stimuli [40], and anchorage-independent growth of a human thyroid papillary carcinoma cell line [41]. On the other hand, transfection of galectin-3 into the prostate cancer cell line LNCaP resulted in suppression of proliferation *in vitro* and slower tumor formation rate in nude mice [42].

A role for galectin-3 in transcriptional control was recently described in thyroid tissue, where the lectin interacts with the thyroid transcription factor TTF-1 resulting in upregulation of its transcriptional activity [43]. These results suggest one mechanism by which galectin-3 can contribute to proliferation of thyroid cancers.

Regulation of apoptosis

A number of studies using gene transfection approaches have provided evidence for a role of intracellular galectin-3 in regulation of apoptosis. These include apoptosis of Jurkat cells induced by anti-Fas antibody and staurosporine [37]; apoptosis of breast carcinoma BT549 cells induced by cis-diamminedichloroplatinum (cisplatin) [44], genistein [45], loss of cell anchorage [46], or apoptotic stimuli induced by nitric oxide [47]; and apoptosis of another human breast carcinoma cell line, Evsa-T, induced by cycloheximide/TNF-α and UVB irradiation [48].

Evidence for a role of galectin-3 in regulation of apoptosis has also been obtained by using galectin-3-deficient mice. When peritoneal macrophages from galectin-3-deficient and wild-type mice were treated with IFN-γ and LPS, a procedure known to induce apoptosis in these cells, galectin-3-deficient cells underwent apoptosis more rapidly [49]. In another model of galectin-3 null mice, increased cell death was observed in chondrocytes at the hypertrophic zone in long bones of embryonic animals [50]. Cell death appeared to proceed through nonclassical apoptotic pathways.

The mechanism underlying galectin-3's anti-apoptotic activity is currently under active investigation. Studies have revealed that galectin-3 is enriched in mitochondria when cells are exposed to apoptotic stimuli, suggesting that it exerts its anti-apoptotic function at these sites [51]. There is evidence that the anti-apoptotic protein Bcl-2 may be involved. Galectin-3 shares significant sequence similarity with Bcl-2 and is shown to bind Bcl-2 *in vitro* [37]; thus, it is possible that galectin-3 functions by interacting with Bcl-2 at mitochondria. It was shown that galectin-3 phosphorylation is required for its anti-apoptotic function [52]. Finally, it was also demonstrated that galectin-3 translocates to the perinuclear membrane [51]. Thus, galectin-3 may be involved in intracellular pathways regulating apoptosis.

A recent study revealed an important role for galectin-3 in macrophage phagocytosis that affects *in vivo* clearance of apoptotic cells [53]. Therefore, this lectin may have an impact on immune processes and tissue modeling, where cellular apoptosis play important roles.

As observed with galectin-1, galectin-3 also has the ability to inhibit cell adhesion by interacting with laminin and other extracellular matrix proteins [54,55]. Though undescribed in these reports, loss of cell adhesion should have consequences in cell survival.

Regulation of the cell cycle

Galectin-3 expression is dependent on the cell cycle, and its expression was initially demonstrated to be upregulated in proliferating cells, preferentially in the nucleus [56]. Thus, the expression of this lectin has been shown to affect the progression of the cell cycle. Transfectants overexpressing galectin-3 responded to the loss of cell adhesion by undergoing G1 arrest without detectable cell death [46]. This effect is associated with

the galectin-3-mediated down-regulation of cyclin E and cyclin A levels (kinases associated with these cyclins are known to be activated in late G1 and S phase of the cell cycle). Galectin-3 is also found to up-regulate the levels of the inhibitory proteins (p21 and p27) for these cyclins. Moreover, retinoblastoma (Rb) protein becomes hypophosphorylated when galectin-3-overexpressing cells lose their cell anchorage. This is consistent with the failure of these cells to enter the S phase, since it is known that Rb is maintained at its hyperphosphorylated state through the S, G2, and most of the M phases. Similarly, galectin-3 expression in BT549 cells results in a different response to a cell cycle regulator, genistein: genistein induces p21 expression in galectin-3-expressing BT549 cells, but not in control BT549 cells [45]. This is consistent with the finding that genistein effectively induces apoptosis without detectable cell cycle arrest in BT549, while it induces cell cycle arrest at the G2/M phase without induction of apoptosis in galectin-3 transfected cells [45].

Other galectins

Galectin-7

Galectin-7 gene is an early transcriptional target of the tumor suppressor protein p53 [57]. Both mRNA and protein are increased after UVB irradiation of epidermal keratinocytes, paralleling p53 stabilization [58]. Keratinocytes transfected with galectin-7 cDNA and thus overexpressing the protein have a higher tendency to undergo apoptosis, suggesting that galectin-7 has a pro-apoptotic function [58]. The mechanism of galectin-7's pro-apoptotic function has been studied in detail using HeLa cell transfectants overexpressing galectin-7 [59]. The results suggest that galectin-7 acts at a common point of apoptosis-signaling pathways and functions intracellularly upstream of JNK activation and cytochrome c release. DNA microarray comparison of the gene expression patterns between galectin-7 and control transfectants have revealed a number of genes whose expression are affected by galectin-7, some of which have been linked to regulation of apoptosis and are redox-related [59]. Thus, galectin-7 might regulate the expression of gene products that modulate the redox status of the cell, resulting in promotion of apoptosis.

Galectin-8

Galectin-8 has been shown to induce apoptosis in human lung carcinoma 1299 cells [60], when added to the cells cultured in plates coated with integrin ligands. This may be related to this lectin's ability to inhibit cell adhesion under the experimental conditions. Additional studies showed that the major galectin-8-interacting protein on the surface of 1299 cells, as well as HeLa and human endothelial cells is $\alpha 3\beta 1$ integrin. Furthermore, transfection of galectin-8 cDNA into 1299 cells significantly reduced colony formation under similar culture conditions, when compared to the number of colonies formed by cells transfected with an empty vector.

Galectin-9

In similar fashion to galectin-1, extracellular mouse galectin-9 induces apoptosis in thymocytes, in lactose-inhibitable fashion [61]. Saita *et al*. [62] found that exogenously added recombinant galectin-9 decreased apoptosis in eosinophils from patients with eosinophilia (E-Eos), whereas it enhanced apoptosis in eosinophils from normal volunteers (N-Eos). Furthermore, recombinant galectin-9 significantly suppressed dexamethasone-induced apoptosis of N-Eos, whereas it did not affect apoptosis of E-Eos. In contrast, this lectin augmented apoptosis induced by anti-Fas antibody in both N-Eos and E-Eos.

Galectin-12

Experimental evidence suggests that galectin-12 is a negative regulator of cell growth. Studies of transfectants ectopically expressing galectin-12 showed that this lectin suppresses cell growth [63] and promotes cell death [64]. In addition, the expression of galectin-12 is dependent on the cell cycle and studies of HeLa cells transfected with galectin-12 cDNA revealed that this lectin induces cell cycle arrest at the G1 phase [63]. The 3′-untranslated region of galectin-12 cDNA contains five AT-rich motifs (ATTTA), which are detectable in many cDNAs coding for proteins of growth regulatory functions, such as oncoproteins and growth factors. These motifs are known to confer instability to mRNA [65,66], and galectin-12 mRNA lability has been observed with a half life of approximately one hour in HL-60 cells induced with phorbol ester (unpublished, Ri-Yao Yang). Analysis of other human galectin cDNAs revealed that the above AT-rich motif was present only in galectin-8. Three to six ATTTA motifs were present in the 3′ flanking regions of various human galectin-8 isoforms.

Conclusion

An image that one obtains with regard to the galectin family is one of multiplicity and diversity, the former from plurality of localization and functionality and the latter from the variety of participating cellular processes of each member, as depicted in Figure 1. In order to appreciate the significance of galectins, one must consider the role of each member individually within the context of cellular compartment and cell cycle status. Then, a consistent pattern is revealed for members of this family. As more mechanistic information is gathered for individual galectins, we anticipate that a greater understanding will result with regard to processes that govern their compartmentalization and functional regulation.

Extracellular functions

Galectins have been shown to bind to a wide spectrum of glycoproteins and they bind to different cell surface glycoligands

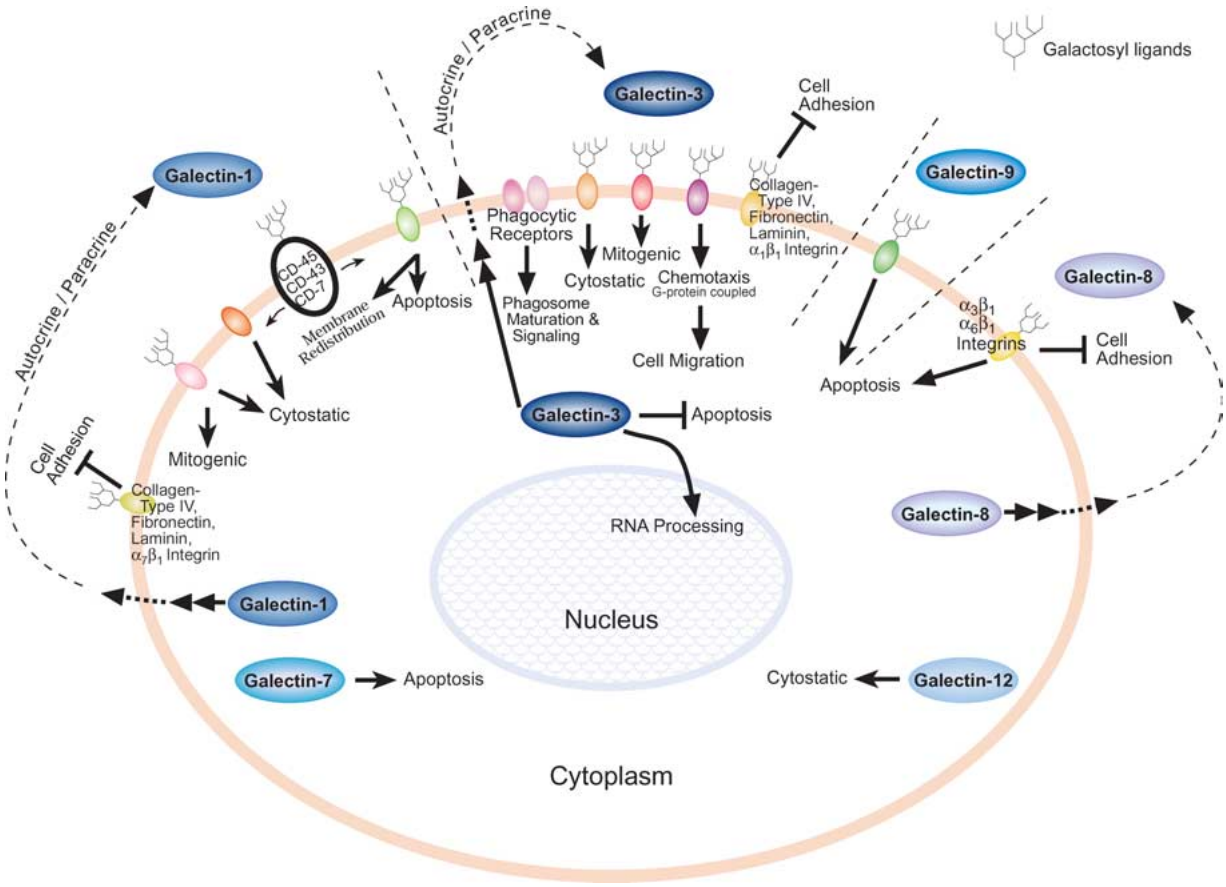

Figure 1. Schematic representation of the known activities of galectins directly affecting cellular homeostasis. Secretion of galectins is depicted by dashed lines terminating with an arrow. Glycosylated membrane receptors are shown with extracellular antennary structures.

in different cell types. They do not have specific receptors, but recognize a group of proteins decorated by oligosaccharides suitable for interaction with these lectins. Thus, galectins may engage a number of cell surface proteins in a given cell type. They may not be potent activators, because it is possible that some cell surface glycoproteins recognized by galectins may not deliver any signals to the cell, and it is also possible that different glycoproteins transmit opposing signals that result in net cancellation. It is probably for this reason that relatively high concentrations of galectins are often required to demonstrate effects and that galectins sometimes have different effects on different cells (*e.g.*, growth promoting in one cell type, while growth inhibiting in another), since different glycoconjugates may be engaged in different cell types.

Two other issues should be addressed in discussing galectins' extracellular functions. First, it is not clear whether galectins are destined to be extracellular proteins. None of the known galectin family members contain a classical signal sequence and some have been shown to be synthesized on cytoplasmic ribosomes and remain in the cytosol. Nevertheless, a number of studies have demonstrated secretion of these lectins. The mechanism underlying the secretion of galectins is not well understood, but plasma membrane targeting and vesicular budding may be critically involved in this process [67]. Recently, galectin-3 has been identified as a component of exosomes in dendritic cells, suggesting an interesting possibility that this lectin and other galectins are secreted in association with exosomes [68]. Second, some galectins appear to require reducing conditions to exhibit biological functions and thus it may be questioned whether they function in the oxidative extracellular environment. This is best exemplified by galectin-1, which is readily inactivated under oxidating conditions and thus its effect is highly dependent on whether reducing agents are present in the media. For this reason, galectins had been initially designated as S-type lectins (*i.e.*, thiol-dependent), which is no longer considered appropriate since many of the biological functions of other galectins are not dependent on reducing agents. In this regard, it has been shown that in the absence of reducing agents, galectin-1 secreted from transfected COS1 cells is in oxidized form with three intramolecular disulfide bonds and lacks lectin activity [69]. This form of galectin-1, but not the reduced form with lectin activity, promotes axonal growth in peripheral nerves [69]. However, it is possible that under certain situations, reducing agents such as glutathione is secreted

by activated cells and thus the microenvironment surrounding the cells may be reducing [70]. Galectin-1 released by the cell may remain reduced under these conditions.

Intracellular functions

The intracellular compartments may be the predominant sites of action of galectins. These proteins have been shown to localize intracellularly, consistent with the absence of a classical signal sequence, as mentioned above, and some (galectin-1, -3) have been ascribed with fundamental intracellular functions, such as pre-mRNA splicing. At present, evidence for intracellular functions are provided by studying the phenotypes of cells made to over-express or under-express a specific galectin, by gene transfection or anti-sense approaches, respectively. In these experiments, the possibility that the observed functions are due to the protein released by the transfectants cannot be excluded. However, in the case of galectin-7, a number of lines of evidence support the fact that the pro-apoptotic activity demonstrated by using galectin-7 transfectants is not attributable to the protein released by the cells and functioning extracellularly [59]. In addition, the fact that some galectins are known to translocate to subcellular structures, such as mitochodria and nuclei, under specific conditions, provide compelling evidence for the existence of intracellular functions. Thus far no specific pattern has emerged regarding intracellular protein targets of galectins or signaling pathways involving galectins. In fact, galectins appear to interact with multiple proteins and contribute to a variety of processes.

Another question is whether intracellular functions of galectins are dependent on the carbohydrate-recognition properties. With the exceptions of cytokeratin [71] and nuclear CBP70 [72], there is no other evidence for intracellular recognition of glycoconjugates by galectins. Saccharides, especially O-linked varieties exist in the cytoplasm and may potentially be ligands of cytosolic galectins. Galectin-3 has been shown to bind Bcl-2 through its CRD and, interestingly, while Bcl-2 is not a glycoprotein, the interaction is inhibitable by lactose [37]. In a similar manner, galectin-3 also self associates through the CRD in lactose-inhibitable fashion [73]. These observations suggest that the carbohydrate-binding sites of galectins may be involved in protein-protein interactions. It has also been noted that the activities of galectin-1 and -3 in inducing pre-mRNA splicing can be inhibited by relevant saccharides [74,75]. Thus, it is conceivable that these activities are dependent on the recognition of nuclear glycoconjugates, or their carbohydrate-binding sites are utilized to interact with other polypeptides involved in pre-mRNA splicing. It is also conceivable that the ancient galectin CRD is utilized as a template in evolution to generate many galectin-related proteins in which the carbohydrate-recognition motif is adapted for protein-protein interactions. However, it has been reported that galectin-3 phosphorylated at serine[6] loses carbohydrate-binding activity [76]. Since it has also been shown that phosphorylation of galectin-3 is essential in this protein's anti-apoptotic function, these observations suggest that the carbohydrate-binding site may not be relevant in some intracellular functions.

Galectins as a family of regulators of cellular homeostasis

This review describes the participation of galectins in regulation of cell growth, apoptosis, and cell cycle. It is evident that other members of the galectin family are also involved in one or more of the above processes. As our knowledge base increases it is likely that this lectin family as a whole can be considered to be regulators of homeostasis. Activities of galectins on cell growth can either be positive or negative depending on cell type and environment. The outcome on cell survival may rely on balancing the activities of specific galectin members, much like coordinating the functions among members of the Bcl-2 family, some of which are pro-, and others anti-apoptotic. Thus, the survival of a variety of cell types may be governed by the levels of expression, subcellular localization and activation status of different galectin family members.

Since maintaining cellular homeostasis is critical in normal physiological processes and perturbation of cellular homeostasis is linked to a variety of pathological conditions, galectins are likely to play important roles in both physiological and pathological processes. For example, mitogenic properties of galectin-1 suggest roles of this lectin in neoplastic transformation and progression as proliferative stimuli during conditions of genetic damage can potentiate cells towards the tumor phenotype. Likewise, mitogenic properties of galectin-3 during cell stress and injury can facilitate the transformation process during neoplastic initiation and contribute to progression. Moreover, the anti-apoptotic activity of galectin-3 is relevant to homeostatic deregulation during cellular transformation, as prolonged cell survival under conditions of cellular and genetic injury can facilitate conversion to the neoplastic phenotype. Furthermore, new information demonstrating additional mechanisms by which galectins can contribute to cellular dysregulation are evident in involvement of galectin-1 in wild type and oncogenic Ras-regulated signaling, and the role for galectin-3 in transcriptional control with TTF-1. It is anticipated that a thorough understanding of galectin biology will become increasingly important in our quest to achieve normal cellular homeostasis and well-being of the organism.

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