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Galectins in cell growth and apoptosis

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Abstract. Fourteen members of the galectin family, proteins with conserved carbohydrate-recognition domains that bind β-galactoside, have been cloned and more are expected to be discovered in the near future. Many aspects of galectin biology have been thoroughly explored, and functional studies have implicated these proteins in cell growth, differentiation and apoptosis, in addition to cell adhesion, chemoattraction and cell migration. In some cases a galectin can either promote or suppress cell growth, depending on the cell types and doses used. Galectin-3 is the only member known so far to inhibit apoptosis, while galectin-1, -7 and -9 promote this cellular process. Galectins can act either extracellularly or intracellularly to exert effects on cell growth and apoptosis.

Key words. Animal lectins; galectins; cell growth; cell cycle; apoptosis.

Introduction

Galectins, a family of animal lectins with conserved carbohydrate-recognition domains that bind β-galactosides, have been found in many species of the animal kingdom, including sponges, nematodes and humans [1]. The crystal structure of the carbohydrate-recognition domains of several galectins have been determined to be a highly conserved tight sandwich fold consisting of two antiparallel β sheets, one 5-stranded and the other 6-stranded, with the sugar binding site formed by the amino acid side chains of the 6-stranded β sheet [2–6]. The occurrence of these proteins early in evolution and the expansion of the family during evolution suggest essential functions for this family of proteins. Indeed, so far galectins have been implicated in cell-cell and cell-extracellular matrix interactions [7], and cell migration [8]. In addition, more and more evidence has emerged recently implicating this family of proteins in cell growth and apoptosis [9], two major themes of normal development and tumorigenesis. Galectins that are involved in these two cellular processes include galectin-1, -3, -7, -9 and -12.

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Galectin-1

Structure and expression

Galectin-1 is a homodimer of two 14-kDa polypeptides. Each subunit consists of almost exclusively one carbohydrate recognition domain (CRD). The crystal structure of galectin-1 was the first to be determined among galectins and consists of one 6-stranded and one 5-stranded antiparallel β sheet [2]. In normal tissues, galectin-1 is expressed in adult muscles, including skeletal and smooth muscle, thymus, lymph node, prostate, spleen, liver, placenta, endothelial cells, skin, testes, olfactory neurons and developing brain [9]. The expression of galectin-1 changes during embryonic development [10]. It is initially synthesized in the trophectoderm of expanded blastocysts immediately prior to implantation. In the postimplantation embryo, the lectin is abundantly expressed in the myotomes of the somites. These changes of expression during development are regulated by DNA methylation of the galectin-1 gene [10–13].

Role in cell growth

Both negative and positive effects have been demonstrated for galectin-1 on cell proliferation. Wells and Mallucci [14] purified a protein with cytostatic activity that is secreted from mouse embryonic fibroblasts (MEFs), and subsequently cloned the complementary DNA (cDNA)

for this protein based on the amino acid sequences of the peptides after tryptic digestion. They designated this protein mGBP for mouse galactoside-binding protein, which is now known as mouse galectin-1. Treatment of unsynchronized cells with recombinant galectin-1 caused cell cycle arrest at the G2 phase, and the addition of galectin-1 to quiescent (G0) MEF prevented serum-stimulated reentry of the cells into the cell cycle. The growth inhibitory effect is not related to the lectin properties, as it is not sensitive to the presence of lactose. A neutralizing monoclonal antibody was further used to confirm the cytostatic activity of the endogenous protein. Addition of this antibody to G0 cells accelerated reentry of the cells into the cell cycle after serum stimulation. This group also found that galectin-1 is secreted by activated T cells, and recombinant galectin-1 inhibits antigen-induced proliferation of T cells [15] as well as the interleukin (IL)-2 independent proliferation of malignant T cells, by arresting them in the S and G2/M phases of the cell cycle [16]. They also treated three human mammary cell lines differing in their oncogenic potentials with galectin-1 and found that in all cases galectin-1 induced a cell cycle block prior to the cells' entry into G2 phase [17]. Overexpression of galectin-1 by transfection, on the other hand, causes transformation of BALB 3T3 fibroblasts [18], a finding that was also confirmed with the rat fibroblast cell line Rat-1 [19]. Another report showed 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 induce cell proliferation in a lactose-inhibitable fashion [20]. Galectin-1 also suppresses the proliferation of freshly isolated mouse thymocytes by modulating TCR signaling and inhibiting IL-2 production [21].

An interesting aspect of galectin-1 is the involvement of its growth-regulating function in neurobiology. Elevated levels of galectin-1 messenger RNA (mRNA) were shown by Northern blot in glioma compared with normal tissue counterparts, and the expression level was found to correlate with states of malignancy, from low-grade astrocytoma to glioblastoma [22]. Transfection of a rat glioma cell line expressing high levels of galectin-1 with a construct that produces antisense galectin-1 mRNA arrests the growth of this cell line, suggesting a growth-promoting role for endogenous galectin-1 [22]. Galectin-1 is also involved in nerve regeneration. Horie et al. [23] isolated a factor secreted from COS-1 cells that enhanced axonal regeneration, and later identified it as galectin-1. Because galectin-1 is expressed in the regenerating sciatic nerves as well as in both sensory and motor neurons, it may regulate initial repair after axotomy. In support of this, application of functional anti-galectin-1 antibodies strongly inhibited axonal regeneration in vivo as well as in vitro. Galectin-1 secreted from transfected COS1 cells is oxidized and lacks lectin activity [24]. This form of galectin-1, but not the reduced form with lectin activity, promotes axonal growth in peripheral nerves after axotomy [23, 24]. Galectin-1 also inhibits the growth of neuroblastoma cells in a carbohydrate-dependent manner and could be an effector in the sialidase-dependent growth control in these cells, since ganglioside GM1 is a ligand of galectin-1, and increased ganglioside sialidase activity leads to elevated cell surface presentation of GM1 [25].

Galectin-1 is also implicated in the conversion of dermal fibroblasts to muscle and myoblasts to the terminally differentiated state. Exposure of clones of dermal fibroblasts to galectin-1 results in the conversion of the cells to myocytes, and treatment of the C2C12 mouse myogenic cell line and primary mouse myoblasts with galectin-1 leads to terminal differentiation [26, 27]. Since growth arrest is virtually a sin qua non of the differentiation process, galectin-1 must somehow arrest the cell cycle in these cells besides affecting other muscle-specific gene expression.

Little is known about the mechanism of growth modulation by galectin-1. Data presented above showed that the effects of galectin-1 on cell proliferation can be either positive or negative, with or without the involvement of its lactose-binding activity. In one case, the site on galectin-1 responsible for its growth inhibitory activity has been partially mapped to include a surface loop (residues 25–30) and two internal β strands, clearly distinct from the sugar-binding site [28]. It is not likely that galectin-1 directly acts on the central cell cycle machinery, and the mediators of the effects of galectin-1 remain to be identified. In a particular case, however, oncogenic H-Ras was identified as an intracellular galectin-1-binding protein, and its transformation activity was shown to be dependent on galectin-1, which appears to direct the anchorage of activated Ras to the cell membrane, where it activates its downstream effector Raf [19]. Overexpression of galectin-1 increased the levels of membrane-associated Ras, Ras-GTP and extracellular signal-related kinase (ERK) activity, resulting in cell transformation that is blocked by dominant-negative Ras. Conversely, antisense galectin-1 RNA inhibited H-Ras(12V) initiated transformation and abolished its membrane anchorage [19].

Role in apoptosis

Exogenously added galectin-1 was first reported to induce apoptosis in activated human T cells and certain human leukemia T cell lines [29]. Resting T cells also bound galectin-1, but did not undergo apoptosis. The mechanism of galectin-1-induced apoptosis appears to be distinct from that triggered by Fas [29], yet the relative importance of galectin-1 versus Fas/Fas ligand in the death of activated T cells in vivo remains to be determined. The

same group also showed that galectin-1 is produced by thymic epithelial cells [30, 31], and similar to activated T cells, certain subsets of thymocytes are also susceptible to galectin-1-induced apoptosis [32]. Exposure of thymocytes to anti-CD3 antibody enhanced galectin-1-induced apoptosis [32], suggesting that galectin-1 may be involved in thymocyte apoptosis mediated by the T cell receptor (TCR), an important process in thymic selection. Similarly, galectin-1 enhances apoptosis induced by TCR engagement in T cell hybridoma and freshly isolated thymocytes, at least in part through antagonizing IL-2 production [21]. While the binding partners for galectin-1 are largely undefined on thymocytes, CD45, CD43 and CD7 are the three major glycoproteins on the T cell surface that bind galectin-1 [33], and galectin-1 regulates CD45-induced signaling in Burkitt lymphoma B cells. Although initial experiments identified CD7 [34] and CD45 [29, 35] as the major mediators of galectin-1-induced apoptosis in T cells, recent work showed that CD45-deficient Jurkat cells exhibit susceptibility to galectin-1, which is indistinguishable from that of their wild-type counterparts [36]. There is also one report showing the involvement of the transcription factor AP-1 and Bcl-2 in galectin-1-induced apoptosis [37]. When mature T cells were cultured in the presence of galectin-1, AP-1 was activated. Treatment of cells before galectin-1 exposure with curcumin, an inhibitor of AP-1 activation, suppressed apoptosis, suggesting that AP-1 activation is required for galectin-1-induced apoptosis. Galectin-1 also inhibits the induction of Bcl-2 by the plant lectin concanavalin A (Con A). It is not known, however, whether galectin-1 treatment alters Bcl-2 expression in the absence of Con A.

Galectin-3

Structure and expression

Independently identified as immunoglobulin E (IgE)-binding protein from rat basophilic leukemia cells [38], Mac-2 antigen from mouse macrophages [39, 40], CBP35 [41], L-29 [42] and L-34 [43] from fibroblasts, galectin-3 is the only member of the family identified so far with the chimeric type of structure: an amino terminal domain composed of tandem repeats rich in glycine and proline residues preceding a single CRD. Hsu et al. [44] showed positive cooperativity in the binding of galectin-3, but not its C-terminal region, to IgE coated on microtiter wells. This suggests self-association of this protein through the amino-terminal region of the molecule, which was later confirmed by Mehul et al. [45]. In the absence of the engagement of its CRD, however, galectin-3 self-associates through both its N- and C-terminal domains [46]. Occupancy of its CRD disrupts C-terminal domain self-association without affecting N-terminal domain interaction [44, 46]. The X-ray crystal structure of the human galectin-3 CRD, in complex with lactose and *N*-acetyllactosamine, has been determined at 2.1-Å resolution. In accordance to the above-mentioned biochemical evidence [46], galectin-3 CRD in complex with lactose and acetyllactosamine does not homodimerize [4], a clear distinction from other galectin CRDs, which form dimers by analogous CRD-CRD interactions regardless of the occupancy of their sugar binding sites [2–6].

Galectin-3 is widely distributed in normal and disease tissues. It is highly expressed in a variety of epithelial cells, dendritic cells and inflammatory cells, especially macrophages [47]. Although T cells normally do not express galectin-3, human T cell lymphotropic virus 1 (HTLV-1) infection induces galectin-3 expression through the viral Tax protein [48]. Galectin-3 expression is also induced in specific types of lymphomas [49], thyroid carcinoma [50–52] and hepatocellular carcinoma [53]. In other kinds of neoplasms, including colon carcinoma [54], breast carcinoma [55], ovarian carcinoma [56] and uterine carcinoma [57], however, galectin-3 expression is downregulated. The levels of both mRNA and protein of galectin-3 are greatly upregulated when quiescent cells are stimulated by serum to reenter the cell cycle, in a manner comparable to other mitogen-activated genes, including the oncogenes c-*fos* and c-*myc* [58].

Like other galectins, galectin-3 is mainly an intracellular protein [59–66]. However, the subcellular localization of galectin-3 could change as cells enter different growth states. For example, in actively proliferating 3T3 mouse fibroblasts, galectin-3 was found in both the cytosol and nucleus [60, 67]. As cells enter quiescence state after serum deprivation or senescence, nuclear localization is lost, and galectin-3 is exclusively a cytosolic protein in these cells [66, 67]. Cowles et al. [67] were the first to show that galectin-3 can be phosphorylated, and that while phosphorylated galectin-3 can be found in both the cytoplasm and the nucleus, unphosphorylated galectin-3 exists exclusively in the nucleus. Recent data showed that the N-terminus of galectin-3 governs the cellular localization of galectin-3 [68]. Deletion of this region abolishes nuclear localization, while fusion of this region with the green fluorescent protein (GFP) directs the localization of the GFP-fusion protein to the nuclei [68]. Despite the differential distribution of phosphorylated and unphosphorylated galectin-3 in the cytoplasm and nuclei [69], phosphorylation does not seem to play an important role in nuclear targeting, as point mutations of serine 6, which account for 90% of galectin-3 phosphorylation [70], did not affect the localization of galectin-3 [68].

Role in cell growth

Growth-related expression and localization of galectin-3 suggest roles of this protein in the regulation of cell pro-

liferation. Indeed, significant evidence has accumulated over the years that supports this notion. Jurkat T cells transfected with galectin-3 exhibited enhanced growth in low serum medium compared to control transfectants [71]. Since apoptosis in both transfectants is not significant under this condition, the difference in cell growth is most likely the result of galectin-3's effect on proliferation. In human prostate cancer, galectin-3 expression decreases with progression of the disease [72–74]. Consistently, transfection of galectin-3 into the prostate cancer cell line LNCaP resulted in suppression of proliferation, and cells ectopically expressing galectin-3 formed tumors at a slower rate than control cells when injected into nude mice [75].

Adding exogenous galectin-3 to cell cultures also affects cell growth and differentiation. For example, in a model for renal epithelial morphogenesis, exogenously added galectin-3 inhibits the cyst enlargement of Madin-Darby canine kidney (MDCK) cells in three-dimensional (3-D) Matrigel, while galectin-3-specific antibodies promote this process [76]. On the other hand, it was found that when cultured in collagen gels, ricin-resistant mutant MDCK cells defective in glycan synthesis, and thus lacking cell surface ligands for galectin-3, form cell cysts with faster growth rates than their wild-type counterparts [77]. Immunocytochemistry analysis of in situ bone marrow sections demonstrated that galectin-3 is present in myelopoietic cells and surrounding stroma, but absent in erythropoietic and lymphopoietic environments [78]. In the presence of galectin-3, proliferation of bone marrow cells induced by recombinant granulocyte-macrophage colony-stimulating factor (GM-CSF) was greatly suppressed, as demonstrated by decreased [^{3}H]thymidine incorporation [78]. Nangia-Makker et al. [79] showed that galectin-3 stimulates capillary tube formation of human umbilical vein endothelial cell in vitro and angiogenesis in vivo. Since endothelial cell morphogenesis is a carbohydrate-dependent process, as it is neutralized by specific sugars and antibodies, these findings suggest that galectin-3 is a mediator of this process [79].

There is also evidence that endogenous galectin-3 is involved in epithelial differentiation. Hikita et al. [80] found that high-cell-density cultures of a clonal intercalated epithelial cell line manifested characteristics of terminal differentiation, while low-cell-density cultures maintained an undifferentiated protoepithelial state. When cells were plated at low density on the extracellular matrix (ECM) of high-density cultures, however, they were converted to the differentiated phenotype, indicating the presence of differentiation-promoting factors in the ECM of high-density cultures. They subsequently purified a protein complex from the ECM responsible for the differentiation promoting activity, which they termed hensin. High-density seeding of cells leaded to the polymerization of hensin and its deposition in the ECM, and only the polymerized form of hensin was able to induce terminal differentiation. They eventually found that galectin-3 is a hensin-binding protein in the ECM, and that galectin-3 is essential for hensin polymerization. High-cell-density cultures secrete galectin-3 into the ECM, where it bundled hensin and allowed the new complex to signal the cells to change to the differentiated phenotype, with permanent growth arrest as part of the process.

Loss-of-function studies also implicate galectin-3 in cell growth. For example, Yoshii et al. [81] transfected galectin-3 antisense cDNA into a human thyroid papillary carcinoma cell line and found that the anchorage-independent growth and saturation density of the clones expressing antisense RNA were significantly suppressed compared with those of control clones. A similar method has been applied to a human breast carcinoma cell line in which expression of antisense galectin-3 resulted in reversion of the transformed phenotype as demonstrated by altered morphology, loss of serum-independent growth, acquisition of contact inhibition and abrogation of anchorage-independent growth [73]. When antisense-transfected cells were introduced into nude mice, tumor growth was found to be significantly suppressed [73].

In most cases, the mechanism behind growth regulation by galectin-3 is not known. With the exception of hensin, which was identified as a binding partner for galectin-3 and cooperates with galectin-3 to promote epithelial differentiation [80], no other ligands have been identified so far that mediate the effects of galectin-3 on cell growth. It is also not known why some cell types respond to exogenous galectin-3 positively, while others respond negatively, in terms of proliferation. In one report, however, it was shown that phosphorylation is required for galectin-3 to mediate anoikis-induced G1 arrest, as nonphosphorylated mutants with serine 6 to alanine (S6A) or to glutamic acid (S6E) mutation failed to evoke G1 arrest when the breast carcinoma cell line BT549 was cultured in suspension [82]. The requirement of galectin-3 phosphorylation for cell cycle arrest is consistent with the observation that galectin-3 in quiescent cells is mostly phosphorylated [69].

Role in apoptosis

Yang et al. [71] showed that galectin-3 ectopically expressed in a human T-cell line protected these cells from apoptosis induced by anti-Fas antibody and the broad-spectrum protein kinase inhibitor staurosporine. The authors also noted sequence similarities between galectin-3 and Bcl-2, a member of a family of major apoptosis regulators. Of particular interest is the presence of the NWGR motif in galectin-3 [71]. The same motif was found in Bcl-2 and is essential for this protein's apoptosis suppression activity and its heterodimerization with Bax

[83]. In addition, galectin-3 is able to bind Bcl-2, and the binding is inhibitable by lactose, suggesting the involvement of the CRD in this interaction [71]. Indeed, galectin-3 devoid of its N-terminal domain is fully able to bind Bcl-2 [71]. The importance of the NWGR motif for the apoptosis-inhibiting activity of galectin-3 has also been demonstrated with a human breast carcinoma cell line. Ectopic expression of wild-type galectin-3 inhibits cis-diamminedichloroplatinum (cisplatin)-induced apoptosis, while expression of galectin-3 with a mutated MWGR motif is ineffective [84].

Subsequent to these findings, galectin-3 has been shown to inhibit apoptosis in different contexts. When denied of cell anchorage, the anchorage-dependent human breast carcinoma cell line BT549 undergoes anoikis, a special case of apoptosis induced by the loss of cell anchorage, which was prevented by ectopic expression of galectin-3 [85]. Galectin-3 probably does this by arresting the cell cycle at G1, a cell cycle phase insensitive to anoikis [85]. This cell line is also sensitive to the anticancer drug genistein. Treatment of control cells with this drug induces apoptosis, while the same treatment of galectin-3-transfected cells causes cell cycle arrest at the G2/M phase of the cell cycle, likely as a result of the elevated levels of the CDK inhibitor p21 in these cells [86]. Transfection of another human breast cancer cell line, Evsa-T, with galectin-3 enhanced cell adhesion and spreading, as well as cell survival upon exposure to apoptotic stimuli such as cytokine and irradiation [87]. It is not known, however, whether the improved survival is a result of enhanced adhesion. It is likely that cells with greater ability to adhere are less liable to stress that could cause anoikis in their counterparts that are less adherent. Moon et al. [88] examined the effect of galectin-3 overexpression on breast cancer cell survival using the liver ischemia/reperfusion metastasis model to further evaluate the role of galectin-3 in metastasis of cancer cells, since studies have suggested that the metastatic outcome is determined to a large extent by the survival of tumor cells in microcirculation. They found that while the majority of control cells died by hepatic ischemia/reoxygenation, nearly all galectin-3-overexpressing cells survived [88]. They further showed that galectin-3 inhibits nitric oxide-induced apoptosis, one of the major cell death pathways induced during hepatic ischemia/reperfusion [88].

In vivo experiments also implicated galectin-3 in apoptosis. Hsu et al. [89] found that compared with their wild-type counterparts, galectin-3-deficient mice consistently accumulate fewer inflammatory cells, especially macrophages, in the peritoneal cavities in response to thioglycollate broth treatment. The relative contribution of cell migration versus cell death to this is not known. However, peritoneal macrophages from galectin-3-deficient mice were found to be more prone to apoptosis induced by lipopolysaccharide and interferon-γ [89].

Much remains to be learned about the mechanism of how galectin-3 inhibits apoptosis, although early studies suggest the involvement of Bcl-2 [71]. There is also evidence [82] that phosphorylation of galectin-3 is required for its inhibition of at least one form of apoptosis, anoikis, induced by the denial of anchorage to anchorage-dependent cells. One recent report showed that galectin-3 translocates to the perinuclear membrane following a variety of apoptotic stimuli and becomes enriched in the mitochondria, where it prevents mitochondrial damage and cytochrome c release [90]. Synexin was identified as a galectin-3-binding protein that mediates this translocation. Downregulation of synexin using an antisense oligonucleotide prevented the translocation of galectin-3 to the perinuclear membranes and abolished its anti-apoptotic activity [90].

Galectin-7

Like galectin-1, galectin-7 is one of the prototype galectins with a single CRD [91, 92]. The crystal structure of galectin-7 has been solved at high resolution, in both free form and as a complex containing galactose, galactosamine, lactose or *N*-acetyllactosamine [5]. The structure shows a fold similar to that of galectins-1 and -2, but has greater similarities to galectin-10. Galectin-7 was originally cloned from human epidermis, and in situ hybridization indicates that this lectin is specifically expressed in keratinocytes at all stages of epidermal differentiation [92, 93]. Galectin-7 expression is greatly suppressed by the SV40 transformation [91] and induced by the tumor suppressor p53 [94]. More recently, galectin-7 has been shown to be rapidly induced in skin keratinocytes exposed to ultraviolet (UV) irradiation in association with p53 stabilization, and the increased levels of the galectin-7 protein was mainly found in sunburn apoptotic keratinocytes [95].

Overexpression of galectin-7 in a squamous cell line induced apoptosis, as demonstrated by increased TUNEL(terminal deoxynucleotidyl transferase dUTP nick end labeling)-positive keratinocytes [95]. Stable transfectants of HeLa and the colon carcinoma cell line DLD-1 are more susceptible to apoptosis induced by mechanistically distinct stimuli [94]. Galectin-7 transfectants displayed upregulated c-Jun N-terminal kinase (JNK) activity upon apoptosis induction, suggesting that galectin-7 functions through JNK to regulate apoptosis. Evidence was also presented that galectin-7 is an intracellular protein and that this lectin is not likely to function extracellularly. Although galectin-7 transfectants exhibited higher JNK activity and altered expression of some genes implicated in apoptosis, it remains to be seen whether they actually mediate galectin-7's function.

Galectin-9

The galectin-9 polypeptide contains two CRDs separated by a linker region [96–98]. There exist several isoforms of galectin-9, with linkers of various lengths [99, 100]. Northern blot analysis showed wide distribution of galectin-9 mRNA, and its expression is developmentally regulated [97]. In situ hybridization revealed an accentuated expression of galectin-9 in liver and thymus of embryonic mice [97]. Galectin-9 immunoreactivity was observed in a variety of tissues in postnatal mice, including thymic epithelial cells [97]. Galectin-9 is now well recognized as a chemoattractant protein for eosinophils [99, 101]. Recombinant galectin-9 was also shown to induce apoptosis in thymocytes but not hepatocytes, in a lactose-inhibitable manner [97].

Galectin-12

Structure and expression

Like galectin-9, galectin-12 is a two-CRD galectin [102]. Galectin-12 cDNA was originally cloned by this group from the human promyelocytic cell line HL-60 [102], and later from human adipocytes by another group [103]. The N-terminal domain of human galectin-12 is highly homologous to the CRDs of other galectins, while the C-terminal CRD of this protein exhibits considerable divergence from the consensus sequence. The mRNA for galectin-12 contains AUGs upstream of the major open reading frame (uAUGs) in its 5′ leader sequence, and several AU-rich elements in its 3′ untranslated region. Whereas less than 10% of eukaryotic mRNAs contain AUG codons within their transcript leader region based on a 1987 survey [104], uAUGs are conspicuously common in certain classes of genes involved in the control of cellular growth and differentiation [104–109]. Similarly, AU-rich elements, the most common determinant of RNA stability in mammalian cells, are rare in eukaryotic mRNAs and are mainly found in the 3′-untranslated regions of mRNAs for protooncoproteins, transcription factors and cytokines [110, 111]. Expression of galectin-12 is high in peripheral blood leukocytes and adipocytes, but very low or undetectable in many tissue and cell lines, except in those of myeloid origin with the potential to undergo terminal differentiation [102, 103]. Galectin-12 expression is upregulated when cells are synchronized at the G1 phase or G1/S boundary of the cell cycle [102]. Similar upregulation of galectin-12 expression was observed when obese animals were treated with troglitazone [103], a ligand for the peroxisome proliferators-activated receptor γ (PPARγ), the pivotal transcription factor in the differentiation and metabolism of adipocytes [112–114]. Immunocytochemistry analysis revealed that galectin-12 is localized in the nucleus, and to a less extent in the cytoplasm in a speckled pattern [103].

Role in cell growth

Structural features of galectin-12 mRNA and correlations between cell cycle synchronization and galectin-12 expression suggest a role for this protein in cell growth control [102]. Indeed, experimental evidence was presented that ectopic expression of galectin-12 suppresses cell growth as indicated by a greatly reduced number of colonies in a colony formation assay [102]. Cell cycle analysis of ectopically transfected cells revealed significant G1 arrest of galectin-12-transfected cells [102]. Induction of galectin-12 expression as cells exit the cell cycle during adipocyte differentiation ([103] and our observations) suggest that galectin-12 may play a role in the permanent growth arrest associated with cellular differentiation.

Role in apoptosis

Upregulation of galectin-12 expression in adipose tissue by troglitazone parallels an increase in the number of apoptotic cells, suggesting the association of galectin-12 with apoptosis. In support of its role in apoptosis, transfection of COS-1 cells with galectin-12 appears to induce cell death. We found, however, ectopic expression of galectin-12 in a panel of cell lines of various origins failed to induce apoptosis [unpublished observations].

Concluding remarks

Since cell growth and apoptosis lie in the center of both normal development and cancer pathogenesis, involvement of galectins in these processes promises a rich and fruitful research field in the future. At present, however, several outstanding questions remain unanswered. In many experiments, recombinant proteins were added to the cell culture. The relevancy of the results from these experiments relies on whether the amount of proteins added is attainable in physiological or pathological conditions. In some cases, the same galectin can either promote or inhibit cell growth, depending on doses and cell types. The exact molecular events that lead to these opposite effects are not known. It is likely that each cell type expresses its unique repertoire of galectin ligands and downstream signaling molecules, giving rise to different outcomes after galectin treatment. Results from ectopic expression of proteins should be more reliable because the proteins are produced by relevant cells and the amount of the proteins produced is within the capacity of the cells. Yet the approach of ectopic expression suffers from possible artifacts associated with overexpression

and cell cloning. Some loss-of-function studies also implicates galectins in cell growth and apoptosis. However, these studies, all involving overexpressing antisense RNAs in cells producing endogenous target proteins, similarly suffer from potential artifacts of cell cloning and the questionable specificity of the antisense approach as a whole. Fortunately, the few lines of galectin knockout mice have produced some data that appear to support the results from in vitro experiments [89]. The recently invented RNA interference (RNAi) with small interfering RNA (siRNA) [115, 116] will certainly be of great value for dissecting the function of individual galectins in view of the high efficiency and specificity this approach is able to achieve.

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