Glycoconjugate Journal 19, 467–477, 2004

Galectin-1: A bifunctional regulator of cellular proliferation

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Galectin-1 has demonstrated a diverse range of activities in relation to cell survival and proliferation. In different circumstances, it acts as a mitogen, as an inhibitor of cell proliferation, and as a promoter of cellular apoptosis. Many of these activities, particularly the mitogenic and apoptotic responses, follow from the interaction of galectin-1 with cell-surface β-galactoside ligands, but there is increasing evidence for protein-protein interactions involving galectin-1, and for a β-galactoside-independent cytostatic mechanism. The bifunctional nature of galectin-1, in conjunction with other experimental variables, makes it difficult to assess the overall outcomes and significance of the growth-regulatory actions in many previous investigations. There is thus a need for well-defined experimental cross-correlation of observations, for which specific loss-of-function galectin-1 mutants will be invaluable. Unsurprisingly, in view of this background, the interpretation of the actions of galectin-1 in developmental situations, both normal and neoplastic, is often very complex. ***Published in 2004.***

Keywords: **galectin-1, cell regulation, growth inhibition, differentiation, cancer, metastasis**

Introduction: Galectin-1 in mitogenesis and apoptosis

Like other lectins, the galactose-binding protein now known as galectin-1 was shown, soon after its discovery, to be an effective mitogen for a range of animal cell types, including spleen cells, lymphocytes and various vascular cells [1–3]. The mitogenic activity of galectin-1 has been seen as having therapeutic potential. A galectin-1 homologue, electrolectin, from the electric eel, displayed an immunomodulatory role in experimental autoimmune myasthenia gravis in rabbits [4], and recombinant human galectin-1 has been shown to have a similar effect on cell-mediated immunity in encephalomyelitis in rats [5]. In both of these cases the lectin appeared to act as a mitogen for some classes of lymphocytes.

Principal targets for galectin-1 binding on lymphocytes are cell-surface glycoproteins with β-galactose residues in the glycan sidechains [6]. These antigenic glycoproteins include CD3, CD4, CD7, CD43 and CD45. CD3 is implicated in signalling, by elevation of cytoplasmic calcium ions, following interaction with the T-cell receptor [7], and a similar response follows from galectin-1 binding to Jurkat T lymphocytes [8]. CD45 is now known to be a tyrosine phosphatase [9], and it has been suggested that inhibition of this enzyme by galectin-1 may increase phosphorylation of the *Lyn* tyrosine kinase, reduce kinase activity, and inhibit signal transmission in lymphoma cells [10]. Galectin-1-mediated signal transmission in Jurkat lymphocytes is inhibited by a tyrosine kinase inhibitor, and involves mobilisation of second messengers from phosphatidylinositol 4,5-bisphosphate [11]. Galectin-1 also activates the ERK/MAP kinase system, in lymphocytes and in hepatic stellate cells [12,13]. These reports suggest that galectin-1 may have both positive and negative effects on signalling kinases. Reporter gene expression in response to galectin-1 indicates that, in lymphocytes, the AP-1 (activator protein-1) and NFAT (nuclear factor of activated T cells) transcription factors are activated. This effect is inhibitable by galactosides, and leads to interleukin-2 expression by the cells [14].

A totally new dimension to this field was introduced when it was observed that galectin-1 could cause apoptosis in activated lymphocytes [15]. This reaction is caused by β-galactoside-dependent binding to CD45 and other cell-surface glycoproteins, and was first demonstrated with relatively high concentrations (20 μM) of galectin-1. Apoptosis may represent the outcome of signalling via an alternative branch of the signalling pathway(s) initiated by CD3 or CD45 [9,16], but a recent report indicates that CD45-deficient lymphocytes are still stimulated to apoptosis by galectin-1 [17]. Characteristic pre-apoptotic responses, such as inhibition of expression of the

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Bcl-2 protooncogene, and caspase activation, are seen in the response of human T lymphocytes to added or endogenously-generated galectin-1 [18]. Many other instances of galectin-1-mediated apoptosis are known, as recently reviewed [19]. The role of galectins as immunomodulators has also been recently reviewed [20]. In several of the early demonstrations of mitogenesis, there was evidence for inhibition of the mitogenic response at supra-optimal concentrations of galectin-1. Though this phenomenon may simply represent a reduced ability of bivalent galectin-1 to cross-link cell-surface receptors as galectin-1 binding approaches saturation, it raised the possibility of growth-inhibitory actions of galectin-1 on animal cells. However, given that, in many cases, net cellular growth may be the aggregate effect of cellular proliferation minus apoptosis, then the apoptotic response to galectin-1 could contribute to apparent growth-inhibitory effects, if it has not been specifically excluded by appropriate investigations.

Galectin-1 as a cellular proliferation inhibitor

Independent evidence for the growth-inhibitory activity of galectin-1 was discovered when galectin research was in its infancy, though the identification of the responsible protein as galectin-1 was not established for another ten years. An autocrine growth inhibitor, produced and secreted by mouse fibroblasts, was first reported by Wells and Mallucci [21]. It inhibited the mouse cell cycle at the S/G2 phase transition. The identity of the protein was not discovered until the corresponding mouse gene was cloned, and shown to be homologous to the human galectin-1 gene [22]. A recombinant form of this mouse galectin-1 (mGBP) was shown to have the same growth-inhibitory activity, which did not appear to depend upon its β-galactoside-binding properties. This latter finding was confirmed when it was shown that the rmGBP was not in fact a functional lectin, being already complexed with an endogenous glycan from the COS cells which expressed it [23]. It should also be noted that mGBP was shown to be active as a growth inhibitor at very low concentrations (0.3–30 nM) [22,24]. This is well below the K_D for dissociation of galectin-1 dimers [25], so it is not unreasonable to regard the monomeric mGBP as being in some respects functionally different from dimeric and bivalent galectin-1 [26].

Wells *et al.* have more recently demonstrated production of mGBP by activated murine T cells, and its role as an autocrine growth inhibitor for these cells [26]. Leukaemic T cells differed from normal lymphocytes in that they were also subject to apoptosis, by a Bcl2/Bax-dependent pathway, following growth arrest by mGBP [27]. Expression of interferon-γ receptor polypeptides, stimulated by mGBP, may render lymphocytes susceptible to apoptosis in response to interferon-γ [28]. In the case of human mammary carcinoma cells treated with mGBP, apoptosis followed cell cycle arrest, though only after a delay of 2–4 days. Three cell lines, differing with respect to tumourigenicity and to the expression of oestrogen and EGF receptors, all demonstrated the same S/G2 cell cycle block in response to mGBP [24]. It is not clear if the apoptosis described in these cases, which follows from a β-galactoside-independent action of mGBP, occurs by a similar mechanism to the apoptotic responses seen by Baum *et al.*, and others, which are dependent upon much higher galectin concentrations and also upon binding to a β-galactoside ligand, as already discussed in the previous section [9,15,16,20].

The lability of the mGBP suggested the possibility that it might be a substrate for a proteinase. Degradation of a negative growth regulator is one hypothetical mechanism for the action of an endogenous growth-promoting cellular proteinase [29,30]. Some circumstantial evidence to support this hypothesis was obtained with human cells. A 14 kDa β-galactoside-binding protein was isolated from human fibroblast-conditioned medium by affinity chromatography. Yields of the protein were very low, but were increased in cultures treated with proteinase inhibitors. The purified protein acted as a fibroblast growth inhibitor in the 0.1–0.7 μM concentration range, although growth stimulation was observed when bovine corneal endothelial cells were treated in the same concentration range [31].

To pursue this line of enquiry, a source of recombinant galectin-1 was needed. Human galectin-1 cDNA from an osteosarcoma cell line was amplified by PCR, and cloned into the pGEX vector, for bacterial expression as a glutathione-S-transferase (GST) fusion protein. This vector and expression system was chosen for reasons of convenience, but fortuitously provided additional evidence for a growth-inhibitory site on galectin-1. The galectin-1 was C-terminal in the fusion protein, and was liberated by proteolytic cleavage with thrombin. Both the fusion protein and the free galectin-1 had lectin activity, with identical agglutination titres against trypsinised rabbit erythrocytes. In contrast, fibroblast growth inhibition was detected only with the free galectin-1, and not with the fusion protein, a clear indication that β-galactoside binding and growth inhibition were independent functions of the protein [32]. Cytotoxicity testing indicated that apoptosis was not occurring, though if it lagged behind the growth-inhibitory effect, as was seen with mGBP and human mammary tumor cells [24], it might not have been detected.

This recombinant galectin-1 preparation displayed a biphasic action on human fibroblasts, with a mitogenic effect, maximal at 70 nM concentration, and inhibitory action at higher concentrations. The mitogenic response was inhibited by lactose, but not the growth-inhibitory effect. In the inhibitory concentration range, it was effective against U2 OS osteosarcoma and HEP2 carcinoma cells, but not against HeLa carcinoma cells. However, there were also some serious limitations inherent in the PGEX-specified galectin-1. Most seriously, the specific activity of the protein was low, requiring concentrations about 15 times higher than those of natural human galectin-1 to give identical growth-inhibiting effects. In addition, the yields of GST-galectin-1 were low, due to poor solubility, and mutated or truncated GST-galectin-1 variants were even less soluble and

correspondingly more difficult to produce in useful amounts [32,33].

Two approaches were used to confirm the separate existence of β-galactoside-binding and growth-inhibitory sites. One of these was to create, or recreate, galectin-1 mutants in which the β-galactoside-binding activity was absent or reduced, and to measure their growth-inhibitory action. Tertiary structure determination and mutagenesis studies have suggested that histidine-45, asparagine-47, arginine-49, tryptophan-69, glutamate-72 and arginine-74 are all involved in, or influence, sugar binding [34–37]. In addition, the observations made with the GST-galectin-1 offered some clues to the location of the growth-inhibitory site itself, which were used to choose additional sites for mutagenesis [33]. The N- and C-termini of the monomer are located close together, on the opposite side of the structure from the galactose-binding site, forming part of the "dimerisation interface" for the self-association of two monomers [35]. It seemed probable that the growth-inhibitory site would be close to, but not within this region, as deduced from the steric restriction of growth-inhibitory activity by the GST domain attached to the N-terminus of galectin-1 in the fusion protein. Surface loops of the galectin-1 structure, which were located close to this interface, were chosen as targets for site-specific mutagenesis.

Despite the limitations imposed by N-terminal extension of the recombinant GST-derived galectin-1, it was considered appropriate to create another "tagged" fusion protein expression system, so that mutants could be purified in the absence of binding to a β-galactoside affinity matrix. The ProEx bacterial expression system was selected, which produced a fusion protein with a hexahistidine sequence and linker peptide (13 amino acid residues) at the N-terminus. This permitted the purification of fusion proteins by nickel ion chelation chromatography [38]. The otherwise-unmodified hexahis-galectin-1 was an effective lectin (endpoint in haemagglutination titre 70 nM). More importantly, it was active as a growth inhibitor (I_{50} for growth inhibition: 0.3 μM), and at least 15-fold more effective than the recombinant galectin-1 cleaved from GST-galectin-1, and was thus active in approximately the same concentration range as the natural galectin-1. This GST-derived galectin-1 retained a dipeptide extension at the N-terminus, whereas the hexahis-galectin-1 had a 13-residue extension. These findings indicate that the chemical nature of the extensions may have a greater effect on growth-inhibitory activity than their size, though a very large extension, such as the entire GST molecule, is clearly inhibitory [32,33]. The concentration range for the growth-inhibitory effect overlaps that seen previously for mitogenic activity with GST-derived galectin-1, which indicates that the growth-inhibitory effect is dominant.

Arginine-49 is located in the S4 β-strand [39], and has been shown to interact with the 4-hydroxyl of the galactose residue, in the three-dimensional structures proposed by Bourne *et al.* and Liao *et al.* [35,39]. The R49G mutant galectin-1 was thus expected to show reduced or altered lectin activity, but in fact it was not significantly different from that of the normal recombinant galectin-1 (haemagglutination endpoint 70 nM). The D47N mutation, also in the S4 β-strand, has been previously reported as lectin-negative [37], and was in this case a very weak lectin (haemagglutination endpoint 7 μM), but the yields of soluble protein were too low to carry out further characterisation of either of these mutants.

The P79R mutation was not expected to be deficient in β-galactoside-binding. It is relatively distant from the galactose-binding site, in a conserved, surface loop between the S6 and F3 β-strands, and it was surprising that it was almost totally devoid of lectin activity (haemagglutination endpoint 34 μM). This could have been caused by disruption of tertiary structure, but it did retain full antiproliferative activity (I_{50} 0.3 μM). This is a clear confirmation that the galactose-binding and growth-inhibitory sites of galectin-1 are largely or wholly independent. This conclusion was confirmed by our experience with a C131S mutation. This is located close to the dimerisation interface, in the F1 surface β-strand (F1). It was produced in low yield, and was totally devoid of lectin activity, but retained most of the growth-inhibitory activity (I_{50} 0.9 μM) of the normal protein. The loss of haemagglutination activity was shown to be due to disruption of the β-galactoside binding site, as dimerisation was unaffected [33]. An earlier analysis of a C131S substitution found a relatively normal β-galactoside binding activity [37].

Preliminary investigations identified the A28R mutation in the F2-S3 surface loop as having greatly reduced growth-inhibitory activity. This observation prompted further mutational substitutions in this region of galectin-1. The K29M mutation retained its galactose-binding activity (haemagglutination endpoint 70 nM), but it had totally lost its ability to inhibit fibroblast growth. The K29T mutant, with a less radical substitution, had the same haemagglutination titre, but was equally devoid of growth-inhibitory activity, and a D27N mutant also demonstrated full lectin activity, but very low growth-inhibitory activity (I_{50} 11 μM). The region of the F2-S3 loop containing A27-K29 is within 8–10 Å of the N-terminus of normal galectin-1. This is consistent with the effect of N-terminal modifications on growth-inhibitory activity. An M1R fusion protein was expressed in high yield, and was fully functional as a haemagglutinin, but was totally devoid of growth-inhibitory activity. Thrombin treatment cleaved the 14-residue hexahis-linker-R1 region. The resulting protein retained haemagglutinin activity, and had also recovered growth-inhibitory activity, though not to the same extent as the wild-type galectin-1 (I_{50} 1.6 μM; [33]). The Figure 1 shows the part of the protein (region c) that has been demonstrated to have a role in the growth-inhibitory activity. The β-galactoside-binding site (region a), and the dimerisation interface (region b) are also indicated. This identification of a growth-inhibitory site is still somewhat tentative, since physically-distant mutational substitutions are also known to affect growth-inhibitory activity. The D103A mutation was located in a surface loop, connecting adjacent antiparallel

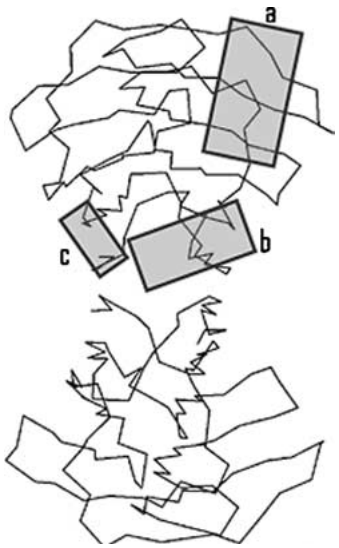

Figure 1. Location of active sites within the galectin-1 structure. This figure is reproduced from Scott and Zhang, *BMC Cell Biology* 2002, **3:3**. (http://www.biomedcentral.com/1471-2121/3/3). It represents the three-dimensional shape of the galectin-1 dimer. The three shaded boxes indicate regions involved in glycan binding (a), dimerisation (b), and cellular growth inhibition (c).

β-strands (F4-F5), and distant from regions a and c. This substitution did not appear to affect the lectin function of galectin-1 (haemagglutination endpoint 70 nM), but growth-inhibitory activity was somewhat reduced (I_{50} 1.3 μM). Unequivocal identification of the growth-inhibitory site waits upon the molecular identification of the molecule, presumably a protein, which interacts with it. This may be possible using a β-galactoside-negative galectin-1 mutant as an affinity ligand, analogous to the method used to isolate CD45 from lymphocyte membranes with natural galectin-1 [10]. What does seem beyond doubt, even at this stage, is that "galectin-negative" ("GN") and "proliferation-negative" ("PN") mutants of galectin-1 can be readily created.

Murine galectin-1, as mGBP, is apparently a much more effective growth inhibitor than the human homologue [22,31,33]. Of eleven amino acid differences between the respective sequences [40], only serine-26 (proline in murine galectin-1) is close to the proposed growth-inhibitory site, and S26P would be a good candidate for future mutagenesis studies on human galectin-1. Murine-human galectin-1 chimaeras could be more effective growth inhibitors for human cells, but it is equally possible that mouse cells may have a higher-affinity receptor for the growth-inhibitory site. It is also possible that there is another, as yet unidentified, part of the galectin-1 structure that contributes to the growth-inhibitory site, and which is different in murine galectin-1.

Oxidation of galectin-1 has been reported to create a molecular variant in which disulphide bonds are formed, and lectin activity is lost. In this form, human galectin-1 can promote axonal regeneration in rat neurites at very low concentrations (optimal at about 30 pM; [41]), and promoted neuronal repair in a subsequent *in vivo* study [42]. In an earlier report, a rat galectin-1 variant, purified from virally-transformed cells, acts as a "transforming growth factor", causing the acquisition of a transformed phenotype which included loss of anchorage dependence, reduced contact inhibition, colony formation in soft agar and tumor formation in nude mice [43]. The mitogenic activity for mouse fibroblasts was optimal at about 0.3 μM. This protein was also devoid of lectin activity, and contained two intramolecular disulphide bonds [44]. There is no information available on the region of the galectin-1 molecule responsible for these activities. It is tempting to speculate that these activities may be related to the non-lectin-dependent properties of galectin-1 reported by Wells and Mallucci, and Scott and their respective co-workers. A recent report indicates that an association between intracellular galectin-1 and the H-Ras oncoprotein promotes membrane localisation of the latter, and concomitant cell transformation, in human and rodent cells [45], but there is as yet little evidence to link this intracellular event with the extracellular actions of secreted galectin-1. Recent work in this area indicates that the Ras-galectin-1 association may influence the choice of intracellular signalling pathway to which Ras activation is coupled. This phenomenon thus has the potential to convert a proliferative response to Ras into other responses, such as survival, senescence or apoptosis [46].

Autocrine galectin-1 enhances the proliferation of human vascular smooth muscle cells. The nature of this response was investigated with a recombinant GST-galectin-1 fusion protein. This protein was not in itself mitogenic, but enhanced DNA synthesis in the presence of serum when adsorbed to the cell substratum. This may represent an effect mediated through cell-extracellular matrix (ECM) links; galectin-1 binds to $\alpha 1\beta 1$ and $\alpha 7\beta 1$ integrins, as well as to several other ECM proteins. It was noteworthy that galectin-1 binding to the ECM was entirely inhibited by lactose, but its binding to the cell was only partly inhibited by lactose, indicating the possibility of multiple cellular binding sites for galectin-1. Galectin-1 also mediates cross-talk between the cell surface and the ECM, modulating cell adhesion and morphology, rearrangement of actin filaments and assembly of the ECM [47–49]. The fusion protein with the GST domain N-terminal to the galectin-1 moiety may not be ideal for experiments of this type, as has already been discussed.

A sialidase active on cell-surface gangliosides regulates the proliferation of a human neuroblastoma cell line in a mechanism involving galectin-1. Removal of terminal sialic acid from the glycolipid gangliosides exposes a terminal β-galactose residue, and binding of galectin-1 brings about cellular growth inhibition, in a manner that has been likened to density-dependent growth inhibition. Sialidase inhibition results in a failure to create galectin-1 binding sites, and consequent loss of growth inhibition [50]. This is noteworthy as a cytostatic activity, with no evidence for apoptosis. These workers have gone on to show that galectin-3 can bind to the same sites, but does not result in growth inhibition. This was ascribed to differing

topologies of galectin binding, but could represent the effect of a site present on galectin-1, but not on galectin-3 [51]. The effects of sialidase inhibition could also influence apoptosis, for which an analogous modulatory response has been identified. Sialylation of terminal galactosyl residues of T cell glycoproteins, by a transferase, may regulate susceptibility to apoptosis [52].

Neurostatin, a glial cell growth inhibitor purified from rat brain extracts, is a galactose-containing glycosphingolipid which is immunochemically related to the glycan side-chains of some cell-surface proteins. It has been suggested that it may mediate cell-cell contact and density-dependent growth inhibition by interaction with selectins or other lectins [53]. It may have functional similarities to the cell-surface, growth-regulatory gangliosides from neuroblastomas [50,51].

In macrophages, endogenous galectin-1 inhibits the generation of nitric oxide by the action of nitric oxide synthase on arginine, and also promotes arginine hydrolysis by arginase. These antiproliferative reactions are inhibitable by lactose [54].

Galectin-1 resembles growth-regulatory agents such as transforming growth factor β in having both positive and negative effects [55]. Another resemblance is the fact that galectin-1 acts as a differentiation factor. It has recently been reported that murine and human dermal fibroblasts begin to differentiate into myoblasts following growth arrest in response to galectin-1 [56,57]. This activity complements the known role of galectin-1 as a mediator of integrin-dependent myocyte-matrix binding during muscle differentiation [58]. A morphologically-similar differentiation of a rat embryonic cell line, induced in response to the FosB oncogene, correlates with the expression of galectin-1 with an eight-residue N-terminal truncation [59]. Galectin-1 has also been implicated in the differentiation of erythroid cells [60]. Development of mature B cells depends upon the binding of the pre-B cells to stromal cells to form a synapse. The surrogate light chain of the pre-B cell receptor forms a protein-protein interaction with galectin-1 bound to galactosyl residues on stromal glycoproteins. This interaction promotes intracellular tyrosine kinase activity and signal transmission with the pre-B cell, leading to cell maturation [61]. This is clearly a different phenomenon from CD3 and CD45-mediated signalling, discussed in the first section. It provides further evidence for a galactose-independent binding site within galectin-1, but there is as yet no evidence to link it to the growth-inhibitory site.

These latter observations invite comparison with proteins which combine a lectin-like functional domain with another, cell-regulatory domain. Bifunctional lectins, in which the lectin domain may serve to specify and localise the site of action of the regulatory domain, have been reviewed [62,63]. Several of the known cytokines are molecules with this potential (*e.g.* interleukins 1α, 1β, 2, 3, 4, 6 and 7, and tumor necrosis factors α and β [64]).

Specific roles for galectin-1 in normal development and differentiation are consistent with observations of its differential expression and localisation during development [13,65,66]. Tumor cell differentiation is discussed in the next section.

Galectin-1 and tumor development

Having considered some cases of the involvement of galectin-1 in tumor cell proliferation, the question of the wider significance of galectin-1 in tumor development is inevitably raised. This topic is considered by other contributors to this volume, but some aspects relate to the actions of galectin-1 already discussed here.

Malignant cell transformation results in changes in the pattern and composition of surface molecules including carbohydrate structures [67], and it also enhances the expression of galectins [68]. Galectins seem to be key factors implicated in the processes of malignant transformation and metastasis in a variety of gastrointestinal tumors, including stomach, hepatocellular and colon cancer, but also in human and murine breast tumors, head and neck cancers, prostate carcinomas, thyroid and skin cancers, ovarian carcinomas and astrocytomas [20].

Metastatic pancreatic cells exhibit moderate to strong galectin-3 immunoreactivity but have been shown to be negative for galectin-1. Strong immunostaining for galectin-1 was observed in most fibroblasts in the stromal strains of desmoplastic tissue in and around the pancreatic cancer mass but not in the pancreatic cancer cells. Messenger RNA and protein analysis of galectin-1 revealed its low abundance in the normal pancreas [69]. Significantly higher levels of galectin-3 have been found in gastric and hepatocellular cancer compared to normal mucosa cells or normal hepatocytes [70,71]. It is believed that, through its apoptotic immunomodulatory properties, galectin-1 could give pancreatic cancer cells the possibility of escape from the cellular immune response while its overexpression in fibroblasts could be responsible for the remodelling of the extracellular matrix in the formation of the desmoplasmic reaction. In this situation, galectin-3 may be an alternative mediator of cancer cell proliferation.

Several studies of colon cancers showed significantly higher levels of galectin-1 and galectin-3 in comparison to the normal mucosa, and their overexpression was associated with advanced tumor stages and poorer prognosis for patient survival [72–74]. In colorectal mucosa, galectin-1 is also predominantly a stromal product and its overexpression is associated with the neoplastic progression of colorectal cancer [74]. On the other hand, studies of 55 colon carcinomas using immunohistochemical galectin fingerprinting suggested that galectins-1, -3 and -4 may be involved in the early stages of human colon carcinoma development, and that galectin-8 is involved in the later stages [75]. In contrast, Lotz *et al*. [76] report finding that galectin-3 levels decrease in colon cancer progression, whilst Hittelet *et al*. [77] report that galectin-1 association with colon cancer is rare. These contradictory reports might be explained by the well-known genetic heterogeneity of tumor cells [78].

Immuno- and lectin-histochemistry studies of galectin-1 and -3 in routinely-fixed sections of two tumor types with poor patient prognosis (neuroblastoma and small lung carcinoma) showed that galectin-3 was frequently present, as opposed to results of tumor tissue culture studies. The presence of galectin-1, as inferred from cytoplasmic staining, coincided with the proliferative activity of tumor cells [79]. A higher tendency toward overexpression of galectin-1 and -3 was found in less differentiated cancer samples [69].

The analysis of the galectin-1 gene expression in two normal thyroid cell lines (FRTL-5 and PC CL3) and in the same cells following transfection with a range of oncogenes that induced different degrees of malignancy and differentiation, has shown that galectin-1 mRNA levels correlated with the expression of the malignant phenotype [80]. A recent investigation of expression of galectin-1 and -3 during fetal thyroid development showed that the absence of galectin-3 from the thyroid cells during fetal development meant that this lectin was expressed "de novo" during malignant transformation of thyroid epithelium, whilst galectin-1 could be considered an oncofetal antigen [81]. Expression of galectin-1 has been shown to be upregulated in thyroid carcinoma-derived cell lines compared to the normal primary cultures and adenoma cells [82]. Galectin-1 at high levels was observed in all thyroid malignancies of epithelial origin tissue, in contrast to normal thyroid and benign thyroid adenomas [83]. High galectin-1 levels were found in papillary carcinomas but not in follicular adenomas or normal tissue [83–86].

In an extension of their work on galectin-1 as a "transforming growth factor," discussed above [43,44], Yamaoka *et al*. [87] showed that the expression of galectin-1 mRNA correlates with the malignant potential of human gliomas and that expression of antisense galectin-1 inhibits cell growth in an experimental rat glioma model. Furthermore, galectin-1 has been found to be strongly expressed in human gliomas and to significantly modulate tumor astrocyte migration *in vitro* [88]. The authors of this paper felt the need to add a cautionary note, to the effect that the simultaneous expression of other members of the galectin family might exert additive or neutralizing effects to the above-mentioned effects of galectin-1.

High grade astrocytic tumors with high levels of galectin-1 expression were shown to be associated with poor patient prognosis [89]. The levels of galectin-1 and -3 expression were found to change during the progression of malignancy in the tumor. Analysis of xenografts from brains of nude mice revealed a higher galectin-1 expression in invasive areas of xenografts than in non-invasive ones. On the other hand, mice grafted with cells expressing low levels of galectin-1 due to stable transfection of an antisense RNA of galectin-1 had longer survival periods compared to mice grafted with cells expressing normal levels of galectin-1. Addition of galectin-1 to culture media increased cell motility levels in human neoplastic astrocytes [89]. These effects are believed to be due to modifications in the organization of the cytoskeleton and increase in small GTPase RhoA expression (the modulator of actin polymerisation/depolymerisation). This results in higher migratory capabilities and increased aggressiveness of tumor astrocytes.

This situation invites comparison with earlier work, from which it has been suggested [68] that the increased expression of lectins by malignant and metastatic cells might play a role in the metastatic process, by mediating cellular recognition and adhesion in organ implantation. In this view, galectin-1 might act both as a lectin, favouring cell adhesion and as a negative growth factor on responsive adjacent cells to facilitate tumor cell invasion. There is data suggesting that galectin-1 might participate in melanoma cell adhesion to laminin, and in this way could modulate invasion and metastasis [90].

Galectin-1 expression has been found to be increased in advanced human uterine adenocarcinoma cells, compared to normal endometrium [91], and also accumulates in the peritumoral stroma associated with carcinoma of the ovary, affecting cancer cell proliferation and adhesion to laminin-1 and fibronectin [92]. The same high expression of galectin-1 is found in high grade bladder tumors compared to normal bladder cells or low grade tumors [93]. In primary prostate carcinoma samples, galectin-1 has also been shown to accumulate in the stroma and associated fibroblasts, but not in intra-epithelial neoplasia or carcinoma cells. Its levels of expression correlated with the aggressiveness of the tumor [94]. Galectin-1 was found to affect matrix mineralization in the osteoblastic response to prostate cancer cells metastasizing into bone [77].

Contrastingly, in renal cell carcinomas, an increase in tumor aggressiveness seemed to be paralleled by a decrease in the level of expression of galectin-1 binding sites rather than by a decrease in galectin-1 expression [95]. HeLa cells which expressed the ovarian cancer antigen CA125 (a giant mucin-like glycoprotein) have been shown to have tenfold more galectin-1 on their surface than non-tumor derived CA125-deficient CHO cells, despite similar galectin-1 expression levels and total binding capacities. It was suggested that CA125 might be involved in the cellular export of galectin-1 [96].

Recent studies, comparing the plasma membrane proteomes of fibroblasts and mammary carcinoma cells, showed that one of the proteins in the "metastatic signature" of the invasive MDA-MB-435 breast carcinoma cells is galectin-1 [97]. Galectin-1 had been shown to mediate the adhesion of MDA-MB-435 breast carcinoma cells to human endothelial cells, which is a key route for capillary invasion and metastatic spread [98]. The observation that galectin-1 is unique to the plasma membrane proteome of invasive carcinoma cells further supports the connection between this lectin and metastasis. In contrast, comparative proteome analysis of human normal (BEAS 2B) and malignant (A 549) lung epithelial cells revealed that galectin-1 expression was decreased rather than increased in the malignant phenotype [99]. Decreased levels of galectin-1 and galectin-1 reactive sites apparently correlate with an increased level of clinically-detectable aggressiveness of head and neck squamous carcinomas (HNSCC) [100], but, in another report, galectin-1

expression patterns correlate with the degree of squamous differentiation in HNSCC [101].

Because galectin was shown to stimulate the growth of vascular endothelial cells [3], it might be possible that it facilitates angiogenesis in tumors in which cells have lost sensitivity to galectin inhibition.

Expression profiling of immortalized human mammary luminal epithelial cells and variants expressing a moderate and high level of erb B2 showed that, out of 6,000 genes analysed, 61 genes were either up or down-regulated. Differentially-regulated genes included those involved in cell-matrix interactions among which were also galectin-1 and -3 [102]. Galectin fingerprinting in 61 human tumor cell lines of different origin (brain, breast, colon, kidney, lung, skin, hematopoieic and urogenital systems) by RT-PCR shows that human tumor cells express mRNA species for other galectins in addition to galectins-1 and -3 [103]. The interpretation of results of experiments in which galectin-1 and -3 are investigated can be unequivocal only when the involvement of other galectins with overlapping or antagonistic functions can be excluded.

The majority of the evidence suggests that galectin-1 does not generally act as a tumor cell growth inhibitor, but there are some contrary indications, in addition to those already cited. Histone deacetylase inhibitors, including n-butyrate and (R)-trichostatin A, induce the expression of galectin-1 in human colon carcinoma and other tumor cells. This results in suppression of cellular proliferation and of the transformed phenotype, enhancement of cell differentiation and, in some cases, increased apoptosis [104,105]. The role of galectin-1 has been confirmed, because transfection with a galectin-1 expression vector leads to the same series of events [106].

Summary and conclusions

A variety of growth-regulatory phenomena have now been ascribed to galectin-1. There are some apparent inconsistencies, but this is not entirely surprising, given the range of experimental techniques, cell types and galectin-1 preparations which have been used. Nevertheless, some generalisations can be made about the several types of cell growth-regulatory effects. Some of these depend upon an interaction with a cellular β-galactoside ligand, and we can distinguish ligands such as the CD3 and CD45 glycoproteins in lymphocytes, and integrins or other proteins involved in cell-ECM links. Both types of ligand are linked to growth-regulatory signalling pathways, but the former may promote either a mitogenic or a cytostatic response, or alternatively lead to growth inhibition followed by apoptosis. In normal cells, stimulation or stasis seems the most common outcome, whereas stasis and eventual apoptosis seems to be favoured in activated lymphocytes, and in some transformed or tumor cells. The interactions with integrins and related proteins seem to depend upon galectin-1 intervention in cell-cell or cell substratum contacts, and have a largely cytostatic outcome.

There is also strong evidence for cell growth-regulatory effects, which are independent of β-galactoside binding. A separate site on galectin-1 can interact with an as-yet unknown ligand, to inhibit proliferation of many normal and some tumor cell types. It is not clear if this pathway can also lead to apoptosis, or if apoptosis is a separate consequence of a simultaneous or subsequent β-galactoside-dependent reaction. At least one other case of a regulatory protein-protein interaction, involving galectin-1, is known.

It should now be possible to make some cross-correlations between different experimental systems. Even in cases where effects are apparently due entirely to the interaction of galectin-1 with β-galactosides, it would be worthwhile to test the effects of both "GN" and "PN" galectin-1 mutants.

Comparisons of the influences of galectin-1 expression on tumor aetiology are similarly complicated by variations in tumor type and experimental approach. Many tumor cells are clearly resistant to the effects of galectin-1 on their growth and survival, and the cell-adhesive properties of the lectin may be more significant, particularly for metastasis, but there is some evidence that galectin-1-mediated growth inhibition or apoptosis in surrounding tissues may influence tumor growth and metastasis. Again, this is an area where loss-of-function mutants of galectin-1 may have some application. There are indications that galectins-1 and -3 are coordinately regulated, and may cooperate in modulating cancer cell proliferation, and there is a need for further studies in this area. Other galectins may interfere with the actions of galectin-1 and -3, and galectin-binding sites may influence both the function and availability of galectins, and need to be considered in parallel with the lectins themselves.

Acknowledgments

The experiments on galectin-1, carried out in the Scott laboratory, were made possible by support from the Cancer Society of New Zealand, the Auckland Medical Research Foundation, and the Staff Research Fund of the University of Auckland.

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