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GALECTIN-3 AS A MULTIFUNCTIONAL PROTEIN

ANNA KRZEŚLAK and ANNA LIPIŃSKA*
University of Łódź, Department of Cytobiochemistry, Banacha 12/16,
90-237 Łódź, Poland

Abstract: Galectin-3 is a 31 kDa member of a growing family of β-galactoside-binding animal lectins. This protein is expressed in a variety of tissues and cell types and is mainly found in the cytoplasm, although, depending on cell type and proliferative state, a significant amount of this lectin can also be detected in the nucleus, on the cell surface or in the extracellular environment. Galectin-3 is secreted from cells by a novel and incompletely understood mechanism that is independent of the classical secretory pathway through the endoplasmic reticulum/Golgi network. Galectin-3 exhibits pleiotropic biological function, playing a key role in many physiological and pathological processes.

Key Words: Galectin-3, Carbohydrate Binding, Non-Classical Secretion, Ligands, Cell Adhesion, Apoptosis, Cancer Progression

INTRODUCTION

Lectins are carbohydrate-binding proteins that have an affinity for specific oligosaccharides [1]. They have been classified into four groups: C-type lectins, P-type lectins, pentraxins and galectins; the latter were formerly known as soluble-type (S-type or S-Lac) lectins [2]. The galectins are a growing family of β-galactoside-binding proteins. A common characteristic of the galectins is the presence of at least one carbohydrate recognition domain (CRD) of about 135 amino acids with an affinity for β-galactosides [1]. The galectins are widely

* Corresponding author, e-mail: annal@biol.uni.lodz.pl

Abbreviations used: AGE – advanced glycation end products; Bcl-2 – B-cell leukemia/lymphoma 2; CBP – carbohydrate binding protein; CD – cluster of differentiation; CHO – chinese hamster ovary cells; CRE – cAMP response element; CREB – CRE binding protein; EHS – murine Engelbreth-Holm-Swarm tumor; Fuc – fucose; GalNAc – N-acetylgalactosamine; HL60 – human leukemia cells 60; IgEBP – IgE binding protein; Lamps – lysosome-associated membrane proteins; LBP – laminin binding protein; m.m. – molecular mass; M2BP – Mac-2 binding glycoprotein; Mac2 – macrophage cell-surface antigen; MAG – myelin-associated glycoprotein; MP20 – membrane protein 20; N-CAM – neural cell adhesion molecule; NeuNAc – N-acetylneuraminic acid; SP1 – stimulatory or specificity protein 1.

distributed in metazoan organisms. To date, 14 members of the galectin family have been identified [3]. The galectins are cytosolic proteins; however, there is abundant evidence for their secretion from the cytosol via nonclassical pathways or translocation to the nucleus or the other cellular compartments [4, 5]. They have been shown to play roles in diverse biological events, such as embryogenesis, adhesion and proliferation of cells, apoptosis, mRNA splicing, bacterial colonization and modulation of the immune response [6-9]. Moreover, galectins play a key role in various pathological states, including autoimmune diseases, allergic reactions, inflammation, tumor spreading, atherosclerosis and diabetic complications [10-12].

In this review, we give a general description of the specialized features of galectin-3 (formerly known as CBP35, Mac2, L-29, L-34, IgEBP, and LBP).

TISSUE AND CELLULAR DISTRIBUTION

Each galectin exhibits a specific pattern of expression in various cells and tissues. Galectin-3 has been detected in activated macrophages, eosinophils, neutrophils, mast cells, the epithelium of the gastrointestinal and respiratory tracts, the kidneys and some sensory neurons [4, 5]. Moreover, galectin-3 displays pathological expression in many tumors, e.g., human pancreas, colon and thyroid carcinomas (discussed in later sections of this review).

Although galectin-3 is predominantly located in the cytoplasm, it has also been detected in the nucleus, on the cell surface or in the extracellular environment, suggesting a multifunctionality of this molecule. It has been shown that in 3T3 mouse fibroblasts, the nuclear *versus* cytoplasmic distribution of this protein depended on the proliferation state of the analysed cells. In quiescent cultures of fibroblasts, galectin-3 (its phosphorylated derivative, pI ~8.2) was predominantly cytoplasmic; however, proliferating cultures of the same cells showed intense nuclear staining for this protein (nonphosphorylated native polypeptide, pI ~8.7) [13]. The intracellular location of galectin-3 is connected with its role in the regulation of nuclear pre-mRNA splicing and protection against apoptosis. On the other hand, its extracellular location on the cell surface and in the extracellular milieu indicates its participation in cell-cell and cell-matrix adhesion (discussed in the context of ligands for galectin-3 and apoptosis).

PROTEIN/GENE STRUCTURE AND CARBOHYDRATE BINDING

Working according to the architecture of galectins, Hirabayashi and Kasai [14] classified this protein family into proto-type, chimera-type and tandem repeat type. Galectin-3 is the sole member of chimera-type family of galectins. Galectin-3 (m.m. 31 kDa) is found in solution as a monomer with two functional domains [15-18]. Galectin-3 is so far unique in the family in having an extra long and flexible N-terminal domain consisting of 100-150 amino acid residues, according to species of origin, made up of repetitive sequence of nine amino

acid residues rich in proline, glycine, tyrosine and glutamine and lacking charged or large side-chain hydrophobic residues [4, 5, 17, 18]. The N-terminal domain contains sites for phosphorylation (Ser 6, Ser 12) [19, 20] and other determinants important for the secretion of the lectin by a novel, nonclassical mechanism [21]. The C-terminus is the carbohydrate recognition domain (CRD), consisting of about 135 amino acid residues; this is what defines the molecule as a galectin. The CRD of galectin-3 displays an identical topology and very similar three-dimensional structure to that reported for the CRD of the homodimeric galectin-1 and -2 [22, 23] with which it shares 20-25% sequence identity. Like galectin-1 and -2, it is arranged in 12 β strands (F1-F5 and S1-S6a/6b) [24].

The structure of the galectin-3 gene is consistent with the multi-domain organization of the protein. The gene for galectin-3 is composed of six exons and five introns (human locus 14q21-22). Exon I encodes the major part of the 5' untranslated sequence mRNA. Exon II contains the remaining part of the 5' untranslated sequence, the protein translation initiation site and the first six amino acids including the initial methionine. The repetitive sequence in the N-terminal half of the gene product is encoded within exon III. Exons IV, V and VI code the C-terminal half of the protein [4, 17] (Fig. 1).

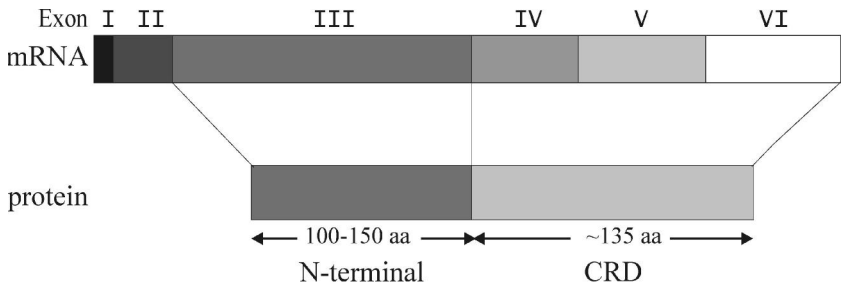

Fig. 1. The organization of the mRNA and structure of galectin-3

Galectin-3 has an affinity for lactose (Lac) and N-acetyllactosamine (LacNAc). The Lac/LacNAc binding site is formed by the β-strands S4-S6a/S6b. The C-4 hydroxyl group of the galactose moiety (Gal) plays a central role in binding, probably accepting hydrogen bonds from the highly conserved residues His 158 and Arg 162, at the same time donating hydrogen bonds to Asn 160 and a water molecule (W1) [24]. The galactose C-6 hydroxyl group also displays this cooperative hydrogen bonding pattern, interacting with Glu 184, Asn 174 and W3. In the case of N-acetylglucosamine (GlcNAc), only its C-3 hydroxyl group makes direct hydrogen bonds to the Glu 184 and Arg 162 of the protein. The only other contacts involving the GlcNAc moiety are mediated through its N-acetyl group; the amide proton is hydrogen bonded through water (W2) to Glu 165 and the methyl group makes a van der Waals contact with the guanidino head group of Arg 186. The van der Waals interaction and the strength of the

hydrogen bond involving the 2 position of the Glc/GlcNAc moiety represent the only significant differences between the Lac and LacNAc complexes, and presumably account for the approximately 5-fold higher binding affinity for N-acetyllactosamine over lactose shown by human galectin-3 [24] (Fig. 2).

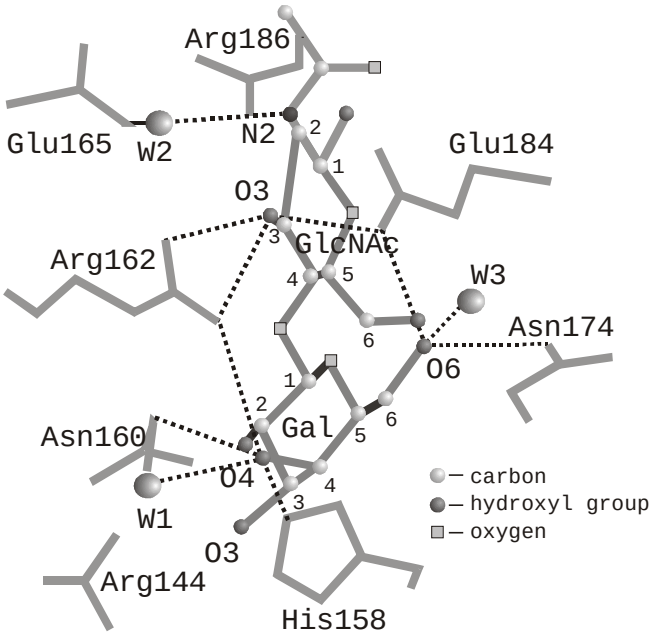

Fig. 2. The interaction of human galectin-3 with the N-acetyllactosamine moiety (for details, see text). The water molecules are labeled W1-W3. Potential hydrogen bonds are shown as dotted lines. The positions of the carbon atoms and the main hydroxyl groups are numbered.

Galectin-3, like most members of the galectin family, acts as a receptor for ligands containing poly-N-acetyllactosamine sequences which consist of many disaccharide units: Gal β1,4 GlcNAc bond to each other by β1,3 linkage. However, galectin-3 appears to have an increased affinity for the more complex oligosaccharides [5, 25]. Extension at the nonreducing end of the disaccharide units with NeuNAcα2, 3 or with GalNAcα1, 3 and Fuc α1, 2 substituents greatly enhances affinity for galectin-3 [5, 6]. Structural and mutagenic studies enabled the identification of contact residues in the galectin-3 CRD responsible for recognition of these more complex carbohydrates [24]. Human galectin-3 was found to have Arg 144, which is well positioned to interact with the GlcNAc moiety or other saccharide residues linked to the O-3 of the terminal galactose [24]. Recently, Hirabayashi *et al.* [25] showed that the N-terminal non-CRD domain contributes to the enhanced affinity of galectin-3 for extended structures of basic recognition units such as Lac or LacNAc. Frontal affinity chromatography analysis revealed that intact galectin-3 showed an on-average 3.8 times higher affinity for oligosaccharides terminated with fucose or sialic

acid residues than its deletion product in which the N-terminal domain was removed by *Clostridium hystolyticum* collagenase digestion.

SECRETION

Galectin-3 is synthesized on free ribosomes in the cytoplasm and lacks any signal sequence for translocation into the endoplasmic reticulum (ER) [5]. Although it does not traverse the endoplasmic reticulum/Golgi network, there is abundant evidence for galectin-3 also having an extracellular location. This protein has been shown to be secreted from cells by a novel, incompletely understood mechanism called ectocytosis, which is independent of the classical secretory pathway through the ER and Golgi system [5, 26-28]. The N-terminus of galectin-3 has been proposed to contain targeting information for nonclassical secretion [21, 29, 30]. It has been shown that a hamster galectin-3 CRD fragment lacking the N-terminal domains, when expressed in transfected Cos cells, is not secreted. Moreover, the addition of the N-terminal segment to a normally cytosolic protein such as chloramphenicol acetyltransferase (CAT) means the fusion protein is efficiently exported from transfected Cos cells [29]. A short segment of the galectin-3 N-terminal sequence comprising residues 89-96 (Tyr-Pro-Ser-Ala-Pro-Gly-Ala-Tyr) has been found to play a critical role in galectin-3 secretion. However, this sequence is not sufficient on its own to cause the direct secretion of the CAT fusion protein, indicating that it is operative in the context of a large stretch of the N-terminal sequence of galectin-3 [21].

Immunohistochemical studies have indicated that the first step in galectin-3 secretion is its accumulation at the cytoplasmic side of the plasma membrane. This step is rate limiting in galectin-3 secretion from macrophages and Cos cells transfected with galectin-3 constructs, and is strongly up-regulated by heat shock and calcium ionophores [26, 29]. It has been suggested that the transfer of protein to plasma membrane domains is mediated through heat shock proteins and other molecular chaperones, which could be a target for the up-regulation of secretion at high temperatures [5]. However, the most important rate limiting step for galectin-3 secretion appears to be the capture of the protein at plasma membrane domains. This is demonstrable by comparing the level of secretion from transfected cells of fusion proteins tagged at the N-terminal with an Lck acylation sequence of the Src protein family of tyrosine kinases and that of wild-type lectin; the former is enhanced relative to the latter [29]. The acylation of Lck is an essential requirement for the retention and functioning of these proteins at the cytoplasmic side of plasma membranes. In transfected cells, the Lck-tagged galectin-3 fusion protein was efficiently acylated and accumulated at the cytoplasmic side of plasma membranes [31]. The next step in galectin-3 secretion is the pinching off of evaginating membrane domains and the release of extracellular vesicles in which galectin-3 is protected against proteolysis. Electron microscopy showed that the vesicles are morphologically heterogeneous and have a small size (up to about 0.5 μm). Under culture

conditions, lectin release from extracellular vesicles was rather fast with a half-life about 1 h [29]. However, isolated vesicles were much more stable, suggesting that the rapid breakdown of vesicles requires factor(s) released by cells. Hughes [5] suggested that a good candidate is one or more members of the phospholipase A2 family that catalyse the hydrolysis of an sn-2 fatty acyl bond of phospholipids and liberate free fatty acids and lysophospholipids. Although this hypothesis is very intriguing, it needs to be checked.

LIGANDS

Owing to its affinity for polylactosamine glycans, galectin-3 binds to glycosylated extracellular matrix components, including laminin, fibronectin, tenascin and Mac-2 binding protein [32-37] (see below Tab. 1). Some cell--surface adhesion molecules, for instance integrins, are also ligands for galectin-3. Preliminary evidence suggests that galectins, through binding to the extracellular domains of one or both subunits of an integrin, may positively or negatively modulate integrin activation, and affect binding with extracellular ligands [6]. A major ligand for galectin-3 on mouse macrophage is the α-subunit of the integrin αMβ2, otherwise known as CD11b/18 [6, 38]. Moreover, galectin-3 also interacts with integrin α1β1 via its CRD domain in a lactose dependent manner [39]. Galectin-3 also seems to be an endogenous cross-linker of the CD98 antigen, leading to the activation of integrin mediated adhesion [6]. The cytoplasmic domain of CD98 binds intracellularly to the cytoplasmic tail of specific integrin β subunits, and promotes integrin activation through as yet incompletely identified intracellular signaling pathways. The association of CD98 and the integrin subunit requires the dimerization of CD98. It is suggested that galectin-3 could promote CD98 dimerization and, indirectly, integrin activation [6].

Despite the specific binding of galectin to glycoconjugates, there are still controversies as to whether galectins facilitate or inhibit cell adhesion. Galectin-3 was shown to reduce the adhesion and spreading of baby hamster kidney epithelial cells on laminin 1 coated wells [35]. On the other hand, purified galectin-3 was shown to promote the adhesion of human neutrophils to laminin [40]. This binary action of galectin-3 may be related to its concentration as well as to the expression level and glycosylation of its cell surface and matrix ligands [6].

Potential ligands for galectin-3 are the lysosomal associated membrane proteins 1 and 2 (Lamp 1 and 2) [41]. Lamps are mainly confined to lysosomal membranes, and are rarely found on the plasma membrane of normal cells. But there is evidence suggesting an increase in their cell surface expression in tumor cells, especially in highly metastatic ones, where they are major carriers for poly-N-acetyllactosamines. Serafian *et al.* [41] demonstrated strong surface binding of recombinant galectin-3 to Lamp-expressing metastasing melanoma cells. These results favor the hypothesis that Lamps could be ligands for cell-

adhesion molecules and could participate in the complex process of tumor invasion and metastasis.

Recent studies have shown that MP20, the lens membrane integral protein and a member of the tetraspanin superfamily, also appears to be a ligand for galectin-3 [42]. It is suggested that MP20 may be a glycoprotein since this protein has a consensus N-linked glycosylation site in one of the extracellular loops, but until now it has not been verified. It is not known exactly what role the MP20/galectin-3 complex could play in the lens. MP20 acts in lens development because point mutations in the MP20 gene cause lens vacuolation and fiber cell disorganisation. It is conceivable that galectin-3 plays an essential role in modulating the ability of MP20 to form adhesive junctions at this critical stage of development [42].

All the above listed ligands for galectin-3 are extracellular matrix or membrane proteins. However, galectin-3 is also known to have an intracellular location and to interact with several proteins inside the cell, i.e., cytokeratins [43], CBP70 [44], Chrp [45], Gemin4 [46], Alix/AIP-1 [9], and Bcl-2 [47]. (the last will be discussed in a later section of this review concerning apoptosis).

It is worth noting that almost all of the mentioned intracellular ligands interact with galectin-3 via protein-protein rather than lectin-glycoconjugate interactions. The only exception is the cytokeratins. Goletz *et al.* [43] have shown that cytokeratins of MCF7 and other human cells carry a novel posttranslational modification, a glycan with a terminal α linked GalNAc. These residues are recognized *in vitro* by mammalian galectin-3. The authors suggest that if similar galectin-cytokeratin associations occur *in vivo*, this might potentially define a new role for cytokeratins as a cytoplasmic anchor for galectins directing or regulating galectin-3 transport and function.

A carbohydrate binding protein with m.m. 70 kDa (CBP70) is a nuclear and cytoplasmic lectin glycosylated by the addition of N- and O-linked oligosaccharide chains [48]. It was first isolated from HL60 cell nuclei [44]. In this cellular compartment, CBP70 interacts with galectin-3 via a protein-protein interaction mediated by the addition of lactose, probably resulting in modification of the galectin-3 conformational structure.

Menon *et al.* [45] used the yeast two-hybrid screen system with murine galectin-3 as bait and the murine 3T3 cDNA library to search for cytoplasmic proteins that might assist intracellular trafficking of galectin-3. They identified a novel protein containing an unusually high content of cysteine (17) and histidine (11) in a total of 311 residues. This protein has been referred to as a cysteine-histidine rich protein – Chrp. Direct interaction between galectin-3 and Chrp was confirmed by immunoprecipitation and *in vitro* binding assays. The confocal immunofluorescence of permeabilized 3T3 fibroblasts showed a predominant perinuclear as well as cytoplasmic location of a novel protein but not nuclear one. Later studies showed that Chrp recognizes the CRD domain of galectin-3 and not the N-terminal repeat sequence [49]. The exact site within the CRD of galectin-3 recognized by Chrp has not been identified yet. However, complex

formation with Chrp does not interfere with the binding of galectin-3 to the polylactosamine glycan of laminin, showing that Chrp and high affinity oligosaccharides utilize separate binding sites on the CRD. The functional significance of the Chrp-galectin-3 interaction remains to be elucidated.

Tab. 1. Ligands for galectin-3.

	Ligand	**Source/cells**	**References**
Extracellular matrix proteins	Laminin	EHS, macrophage, placenta	32, 35, 37
	Fibronectin	Foetal	35
	Tenascin	Brain	36
	M2BP	Brain	33, 34
Membrane proteins	Integrins:		
	αM/β2(CD11b/18)	Macrophage	38
	α1/β1	Adenocarcinoma	39
	N-CAM	Mouse brain	36
	L1	Mouse brain	36
	MAG	Mouse brain	36
	LAMP-1,2	Ubiquitous	38, 41, 51
	MP20	Rat lens	42
	CD98	Human T lymphoma Jurkat cells	38
Intracellular proteins	Cytokeratins	HeLa, MCF-7	43
	Chrp	Murine 3T3 fibroblasts	45, 49
	CBP70	HL60	44
	Alix/AIP-1	Human T lymphoma Jurkat cells	9
	Bcl-2	Human T lymphoma Jurkat cells	52
	Gemin-4	HeLa	46
Others	AGE	Ubiquitous	53-55

Nuclear galectin-3 can interact with Gemin-4, a component of a macromolecular complex containing approximately 15 polypeptides, among them SMN (survival of motor neuron) protein, Gemin-2, Gemin-3, some of the Sm core proteins of snRNPs and other as yet unidentified proteins. These complexes are implicated in processes directly or indirectly related to pre-mRNA splicing. The identification of Gemin-4 as an interacting partner of galectin-3 provides strong evidence that this galectin can play a role in splicesome assembly *in vivo* [9, 46]. The yeast two-hybrid method screen of a Jurkat cell cDNA library using galectin-3 as a bait allowed the identification of another galectin-3 binding protein. It is a human homologue of ALG-2 linked protein x (Alix) or ALG-2 interacting protein-1 (AIP-1) [9]. This protein interacts with ALG-2, a calcium-binding protein necessary for cell death induced by different stimuli. AIP-1

cooperates with ALG-2 in executing the calcium dependent requirements along the cell death pathway [50]. Alix/AIP-1 contains a proline, glycine, alanine and tyrosine rich sequence in the C-terminal region, which is highly homologous to the tandem repeat sequence in the N-terminal part of galectin-3 [9].

Vlassara *et al.* [53] were the first to propose that galectin-3 is one of the AGE receptors. Galectin-3 was identified as an AGE-binding protein in macrophages, astrocytes and umbilical vein endothelial cells [54]. Zhu *et al.* [55] characterized the functional aspect of galectin-3 as an AGE receptor by using CHO cells overexpressing human galectin-3. The results clearly showed that AGE binds to galectin-3 CHO cells in a specific and saturable manner, followed by endocytosis and subsequent lysosomal degradation. Further experiments showed that acetylated and oxidized low density lipoproteins also undergo receptor-mediated endocytosis by these cells. Therefore, galectin-3 is likely to play an important role in the formation of atherosclerotic lesions *in vivo* by the modification of the endocytic uptake of AGE and by modified low density lipoproteins [55].

GALECTIN-3 AS AN INHIBITOR OF APOPTOSIS

There is a series of pieces of evidence showing the involvement of galectin-3 in the inhibition of apoptosis. Cells with galectin-3 overexpression display increased resistance to the apoptotic stimuli induced by the anti-Fas antibody, staurosporine (chemotherapeutic reagent), tumor necrosis factor, radiation and nitric oxide [47, 52, 56-58]. Galectin-3 was found to have a significant sequence similarity with the Bcl-2 protein, a well-known suppressor of apoptosis. The lectin contains a four amino acid motif, Asn-Trp-Gly-Arg, which is a highly conserved sequence within the BH1 domain of the Bcl-2 family proteins and is crucial for Bcl-2 protein function in the inhibition of programmed cell death [47, 52]. Akahani *et al.* [47] showed that an amino acid substitution of Gly to Ala at position 182 in this motif of galectin-3 prevents its anti-apoptotic activity. The four amino acid motif in Bcl-2 is critical for Bcl-2/Bcl-2 homodimerization and Bcl-2/Bax heterodimerization [59]. Yang *et al.* [52] demonstrated that galectin-3 can interact with Bcl-2 in a lactose-inhibitable manner. This finding is very surprising since Bcl-2 is not a glycoprotein. The authors suggested that the Asn-Trp-Gly-Arg motif is present within the carbohydrate recognition domain in galectin-3, and is closely involved in interaction with Bcl-2. Lactose binding to galectin-3 may induce a conformational change in the critical region of this protein, which prevents its interaction with Bcl-2.

The molecular mechanism by which galectin-3 regulates apoptosis induced by different agents remains to be elucidated. However, it is possible that this lectin can replace or mimic Bcl-2 protein. Bcl-2 is a mitochondrial protein located on the outer membranes. It regulates apoptosis by blocking the release of cytochrom c from the mitochondria [60, 61]. Moon *et al.* [58] showed that galectin-3 inhibition of nitrogen free radical-mediated apoptosis in human breast carcinoma BT549 cells involved the protection of mitochondrial integrity, the

inhibition of cytochrom c release and the activation of caspase. Thus, galectin-3 appears to be a mitochondrial-associated apoptotic regulator additional to Bcl-2 [56, 58]. Recent studies have demonstrated that galectin-3 translocates into the mitochondrial membrane following a variety of apoptotic stimuli [62]. Galectin-3 is enriched in the mitochondria, where it prevents mitochondrial damage and cytochrom c release. Such a location of galectin-3 is regulated by galectin-3 interacting proteins. One of these proteins was identified as a synexin, a 51 kDa member of the annexin family of proteins, which can bind to phospholipid membranes. Synexin down-regulation abolished the anti-apoptotic activity of galectin-3 suggesting that translocation to the mitochondrial membranes is crucial for galectin-3 function.

Overexpression of galectin-3 also prevents human breast carcinoma BT 549 cells from undergoing anoikis, a specific form of apoptosis caused by loss of epithelial cell-matrix interactions [63]. It is suggested that galectin-3-mediated inhibition of anoikis may result from its ability to induce cell cycle arrest at late G_1 through the expression modulation of cyclins and their inhibitors. Galectin-3-mediated G_1 arrest involves down-regulation of G_1-S cyclin levels (cyclin E and cyclin A) and up-regulation of their inhibitory protein levels ($p21^{WAF1/CIP1}$ and $p27^{KIP1}$). Galectin-3 also induces cyclin D_1 expression (an early G_1 cyclin) and its associated kinase activity in the absence of cell anchorage. Kim ***et al.*** [63] suggested that galectin-3 induction of cyclin D_1-associated kinase activity may help cells pass the apoptosis-sensitive point in early G_1. Similarly, galectin-3 mediated down-regulation of cyclin E and A expression may be involved in cell cycle arrest at an anoikis-resistant point.

Apart from cell cycle arrest at late G_1 in response to loss of cell adhesion, galectin-3 influences G_2/M arrest of BT 549 cells following genistein (4,5,7-trihydroxyisoflavone phytoestrogen) treatment [64]. Lin ***et al.*** [64] showed that genistein effectively induces apoptosis without detectable cell cycle arrest in BT 549, a human breast epithelial cell line which does not express galectin-3 at a detectable level. In galectin-3 transfected BT 549 cells, genistein stimulates cell cycle arrest at G_2/M phase without apoptosis induction. Genistein induces $p21^{WAF/CIP1}$ expression in galectin-3-expressing BT 549 cells but not in control BT 549 cells undergoing apoptosis.

At present, the way galectin-3 regulates expression of the cyclins and their inhibitors is not entirely clear. Galectin-3 was shown to be a nuclear matrix protein that binds to RNA and single-stranded DNA [65] and identified as a splicing factor in the cell-free splicing assay [66]. Thus, it is possible that nuclear galectin-3 may directly modulate gene expression through regulation of transcription and/or mRNA splicing. The results of recent studies by Lin ***et al.*** [67] seem to confirm this possibility. The authors found out that galectin-3 induces cyclin D_1 promoter activity in BT 549 cells through multiple ***cis***-elements, including SP1 and CREB binding sites. They also showed that galectin-3 induction of the cyclin D_1 promoter activity may result from the

enhancement/stabilization of nuclear protein-DNA complex formation at the CRE site of the cyclin D_1 promoter.

Galectin-3 phosphorylation is required for its anti-apoptotic activity and anti--anoikis activity [68]. Human galectin-3 is phosphorylated at Ser 6 by casein kinase I. Previously, phosphorylation of galectin-3 was shown to regulate its carbohydrate recognition [20]. Ser 6 phosphorylation of human galectin-3 significantly reduces its binding ability to ligands, e.g. laminin and asialomucin, while dephosphorylation fully restores the sugar binding activity [20]. Yoshii *et al.* [68] demonstrated that galectin-3 phosphorylation can also influence its other biological activities. Ser 6 mutation resulted in a relative decline in the level of galectin-3's ability to protect cells against *cis*platin-induced cell death and poly(ADP-ribose)polymerase from degradation when compared with wild type galectin-3.

GALECTIN-3 IN CANCER PROGRESSION

Thyroid

Galectin-3 expression in normal and neoplastic human thyroid tissue has recently been reported on by several investigators [69-80]. These studies demonstrated the overexpression of galectin-3 in thyroid carcinomas, while its expression in normal tissue and adenomas was absent or weak. In addition, galectin-3 mRNA was observed in all malignant thyroid lesions, while in normal and non-malignant tissues, it was not detectable [72]. Galectin-3 expression in malignant thyroid cells was detected by immunohistochemistry on both cytological paraffin samples (cell blocks) obtained by fine-needle aspiration biopsy (FNAB) and histological sections. Galectin-3, when expressed, was predominantly found in the cytoplasm of follicular and parafollicular cells; a nuclear location was also sometimes observed. Kawachi *et al.* [74] reported differences in galectin-3 expression between the primary lesions of papillary carcinomas with metastases and those without metastases; the expression of galectin-3 was significantly higher in the former (score: 1.613±0.742) than in the latter (score: 1.308±0.782). A trend towards a stronger expression of galectin-3 in the observed stages of medullary thyroid carcinoma (with regional lymph node metastases) was also observed [73]. On the other hand, expression of galectin-3 in the lymphatic metastases of papillary carcinoma appeared to be significantly lower than in corresponding primary lesions [74]. In the case of medullary thyroid carcinoma, galectin-3 expression was also reduced markedly in lymph node metastases as compared to corresponding thyroid tumors [78]. The published observations permit the conclusion to be drawn that cytoplasmic galectin-3 expression is a phenotype associated with malignant transformation and progression toward metastatic potential. Yoshii *et al.* [75] showed that antisense inhibition of galectin-3 expression in thyroid papillary carcinoma cells resulted in a marked reduction of the malignant phenotype. It is possible that the transforming function of galectin-3 may result from its action as an anti-

apoptotic molecule. Recently, Takenaka *et al.* [80] proved that overexpression of galectin-3 in transfected galectin-3 cDNA normal thyroid follicular cells leads to the acquisition of the malignant phenotype. Moreover, Takenaka *et al.* [80] tried to identify the genes that are associated with overexpression of galectin-3. The genes with increased expression include: *retinoblastoma (RB), proliferating cell nuclear antigen (PCNA) and replication factor C (RCF),* all of which are involved in the G_1-S transition of the cell cycle. Therefore, the possibility of galectin-3 involvement in the cell cycle regulation exists.

From a biochemical point of view, the evaluation of cytoplasmic galectin-3 expression in epithelial cells isolated from FNAB should serve to make a differential presurgical diagnosis between benign follicular adenomas and differentiated carcinomas (i.e., papillary and widely and minimally invasive follicular carcinomas). Routine use of this analysis can lead to the better selection of patients who really require surgery.

Digestive system

Using Northern and Western blotting analysis, *in situ* hybridization and immunohistochemistry, Barberat *et al.* [81] studied the expression of galectin-3 in primary pancreatic cancers and in tumor metastases in comparison to normal pancreas. The results showed that galectin-3 was strongly overexpressed at the mRNA and protein level in human pancreatic cancer compared to the level of its expression in normal human pancreas cells. However, no relationship was found between galectin-3 expression and the tumor stage. On the other hand, metastatic pancreatic cancer cells in lymph nodes and in liver showed strong galectin-3 immunoreactivity, indicating that galectin-3 might have an impact on metastasis formation.

The results of studies by Hsu *et al.* [82] demonstrated that normal hepatocytes do not express galectin-3, but that this protein can be present in hepatocellular carcinoma (HCC). The investigation revealed that galectin-3 expression in HCC is independent of whether the patient had prior hepatitis B virus (HBV) infection. However, the authors established that lectin expression in HCC can be positively influenced by HBV infection through a mechanism that may include the transactivation of the galectin-3 gene promoter. It was found that focal regenerating nodules of cirrhotic tissue also express galectin-3. Abundant expression of galectin-3 in rapidly proliferating hepatocytes may be a result of a high mitotic index or, alternatively, it is possible that these cells indicate early neoplastic events [82].

Conflicting data was published regarding the expression of galectin-3 in the human colonic mucosa and colonic tumors. Some investigators found decreasing galectin-3 levels in colon carcinoma progression [83, 84], whereas others presented opposite results [85-87]. Castronovo *et al.* [83] and Lotz *et al.* [84] found low levels of galectin-3 mRNA in human colon cancer tissues relative to the levels in normal adjacent colonic mucosa. Castronovo *et al.* [83] indicated that the steady-state mRNA level of galectin-3 was down-regulated by about

50% in 18 of 21 human colon carcinomas compared with the level in their corresponding normal colonic mucosa. Moreover, Castronovo *et al.* [83] stated that patients with Dukes' C and D tumors had a lower ratio of primary tumor galectin-3 mRNA to normal tissue mRNA than did patients with Dukes' B tumors. Lotz *et al.* [84] observed a 5- to 10-fold decrease in galectin-3 mRNA level as well as a significant reduction in the amount of protein expressed in the cancer compared with the normal colonic mucosa. This was accompanied by a translocation of the protein from the nucleus to the cytoplasm during neoplastic progression. On the other hand, Irimura *et al.* [85] found a higher content of galectin-3 in advanced stage of Dukes' D colorectal cancer than in specimens of the early-stage disease. Schoeppner *et al.* [86] demonstrated that normal mucosa distant from areas of neoplasia was characterized by weak or negative expression of galectin-3. They indicated that cytoplasmic expression of galectin-3 correlated with progression from adenoma to carcinoma.

Schoeppner *et al.* [86], similarly to Irimura *et al.* [85], stated that galectin-3 expression in invasive cancers varied according to the stage in Dukes' scale. Furthermore, when metastases were compared with the primary tumors from the same patients, distant metastases expressed a high level of galectin-3 relative to the level in the primary tumors from which they arose.

Sanjuan *et al.* [87] separately analysed both cytoplasmic and nuclear galectin-3 expression in this tumor model. They observed that normal colonic mucosa usually strongly expressed galectin-3 both in the nucleus (100% of cases) and in the cytoplasm (77%). Nuclear and cytoplasmic expression was significantly down-regulated in adenomas (60%, 16%, respectively), whereas cytoplasmic expression of galectin-3 increases in the carcinomas again (64%) although it usually did not reaching the level of normal mucosa expression. Nuclear expression was similar to that seen in adenomas (48%) but lower than in the normal mucosa. Contrary to the normal mucosa pattern in which cytoplasmic and nuclear expression of galectin-3 was always associated, the cytoplasmic expression of galectin-3 in carcinomas was frequently observed in cells with negative nuclear staining. A correlation between increased levels of galectin-3 expression and shorter survival periods, particularly in the case of patients with Dukes' A and B colon tumors, was observed [86-88].

To more directly establish the role of galectin-3 in colon cancer metastasis, human colon cancer cell lines with high metastatic potential and high levels of galectin-3 were stable transfected with a plasmid designed to express antisense galectin-3 mRNA. Conversely, colon cancer cells with low metastatic potential and low levels of native galectin-3 were transfected with a plasmid containing the complete coding sequence of galectin-3 mRNA [89]. Down-regulation of galectin-3 expression by antisense transfection resulted in a significant decrease in liver colonizing ability, whereas up-regulation of galectin-3 increased metastatic potential. Moreover, the alterations in galectin-3 levels by the expression of sense or antisense galectin-3 constructs resulted in parallel changes in the level of Muc 2 mucin – a major ligand for galectin-3 [90].

Taken together, these results provide strong evidence that galectin-3 plays a role in the ability of colon cancer cells to metastasize to distant sites.

Breast

It was shown by immunohistochemical methods that normal breast tissue expressed a high level of galectin-3 and that the expression of this protein was down-regulated in breast cancer [91, 92]. The reduced expression of galectin-3 was associated with increasing histologic grade, and thus with the acquisition of invasive and metastatic potential which possibly resulted from reduced extracellular matrix binding and increased cell motility [92].

Different results concerning the role of galectin-3 in breast cancer malignancy were obtained in studies using cell lines. Honjo *et al.* [93] determined that the blocking of galectin-3 expression in highly malignant human breast carcinoma MDA-MB-435 cells led to the reversion of the transformed cellular phenotype and to significant suppression of tumor growth in nude mice. So they suggested that the expression of galectin-3 is necessary for the maintenance of the transformed and tumorigenic phenotype of MDA-MB-435 breast carcinoma cells.

Furthermore, Song *et al.* [94] provided evidence that galectin-3 enhances the metastatic potential of human breast carcinoma BT 549 cells. It was established that galectin-3 in tumor cells can play the role of survival factor against cytotoxic, reactive nitrogen and oxygen species such as NO and $ONOO^-$ possibly through the Bcl-2-like anti-apoptotic function of galectin-3.

Prostate

Immunohistochemical and Western blotting analysis showed a generally reduced expression of galectin-3 in prostate cancer relative to the level in normal human prostate tissue [95, 96]. Subcellular expression of galectin-3 in a collection of 145 primary human prostate carcinomas was studied using immunohistochemistry by Van den Brûle *et al.* [97]. They demonstrated a clear change in the location of galectin-3 in prostate carcinoma cells as compared with non-tumor cells. In general, normal glandular cells expressed galectin-3 in both the nucleus and cytoplasm. In a subset of malignant lesions, galectin-3 was expressed in the cytosol but was generally excluded from the nucleus. Such a distribution of galectin-3 was correlated with disease progression. These results suggest that galectin-3 might have anti-tumor activities when present in the nucleus, whereas it could favor tumor progression when expressed in the cytoplasm [97].

Head and neck

Choufani *et al.* [98] studied the expression of galectin-3 and the expression of ligands for this lectin in 75 cases of head and neck squamous cell carcinomas (HNSCCs) and in 40 normal tissue specimens. The results showed that HNSCCs exhibited a significantly lower amount of galectin-3 and its ligands than their corresponding normal counterparts. A decrease in the extent of galectin-3

expression correlates with an increasing level of clinically detectable HNSCCs aggressiveness. Later studies confirmed the correlation of galectin-3/galectin-3 ligand levels with a low differentiation status which is known as an indicator of the recurrence rate in HNSCCs [99]. Recently, Lefranc *et al.* [100] showed that rapidly recurring craniopharyngiomas also have a significantly lower level of expression of galectin-3 than nonrecurring or slowly recurring cases. On the other hand, the level of expression of galectin-3 is correlated highly and positively with the level of apoptosis in human cholesteatomas [101]. Cholesteatoma is a benign disease characterized by the presence of an unstrained growth and the accumulation of keratin debris in the middle ear cavity. Cholesteatoma can invade neighboring tissues and often recurs even after surgical resection. The level of apoptosis is an indicator for the prediction of the recurrence. It is suggested that an up-regulation of galectin-3 expression, which is associated with pronounced apoptotic activity, could have a physiologically protective effect against the substantial apoptotic features occurring in recurrent cholestestomas [101].

Using immunohistochemical methods, Honjo *et al.* [102] analyzed the intracellular expression of galectin-3 in 77 tongue specimens (54 squamous cell carcinomas and 23 specimens of distinct normal mucosa). They reported that the nuclear expression of galectin-3 markedly decreases during the progression from normal to cancerous states, while cytoplasmic expression increased. The authors suggested that translocation of galectin-3 from the nucleus to the cytoplasm during neoplastic progression may serve as a prognostic factor for tongue cancer patients [102].

Tumors of the nervous system

There is a relationship between the level of expression of galectin-3 and the level of malignancy in human gliomas [103-105]. Bresalier *et al.* [103] showed that normal brain tissue and benign tumors did not express galectin-3 but anaplastic astrocytomas (grade 3) and glioblastomas (grade 4 astrocytomas) respectively exhibit intermediate and high expression. Moreover, these authors reported a more significant expression of galectin-3 in metastases than in the primary tumors from which they derived [103]. Different results were obtained by Gordower *et al.* [104]. They showed that the level of galectin-3 expression significantly decreases in the majority of tumor astrocytes, from low to high grade astrocytic tumors. However, the authors suggested that human astrocytic tumors are very heterogenous, and in spite of the general decrease in the level of galectin-3 expresssion, some tumour cell clones express a higher level of galectin-3 with increasing level of malignancy [104]. A further study suggested that galectin-3 is involved in tumor astrocyte invasion of the brain parenchyma, since its expression is higher in the invasive parts of xenografted glioblastomas than in their less invasive parts [105]. This is in accordance with *in vitro* migration results. Camby *et al.* [105] showed that *in vitro* galectin-3 stimulates

migration (and therefore, presumably malignancy) in the case of cell lines originating from high-grade astrocytic tumors.

CONCLUDING REMARKS

The results of many studies indicate that galectin-3 is a multifunctional protein engaged in different biological events. The abundance of information concerning galectin-3 expression and its ligands enables us to see mechanisms of basic cellular processes such as adhesion, proliferation, signal transduction, mRNA splicing and apoptosis in a new light. It is possible that in the near future, galectin-3 may become an attractive target for the development of new strategies in the diagnostics and treatment of some diseases especially cancers.

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REFERENCES

1. Barondes, S.H., Cooper, D.N.W., Gitt, M.A. and Leffler, H. Galectins. Structure and function of a large family of animal lectins. **J. Biol. Chem.**. 269 (1994) 20807–20810.
2. Drickamer, K. and Taylor, M.E. Biology of animal lectins. **Annu. Rev. Cell Biol.** 9 (1993) 237–264.
3. Kasai, K. and Hirabayashi, J. Galectins: a family of animal lectins that decipher glycocodes. **J. Biochem.** 119 (1996) 1–8.
4. Hughes, R.C. The galectin family of mammalian carbohydrate-binding molecules. **Biochem. Soc. Transact.** 25 (1997) 1194–1198.
5. Hughes, R.C. Secretion of the galectin family of mammalian carbohydrate-binding proteins. **Biochim. Biophys. Acta** 1473 (1999) 172–185.
6. Hughes, R.C. Galectins as modulators of cell adhesion. **Biochimie** 83 (2001) 667–676.
7. Rabinovich, G.A., Riera, C.M., Landa, C.A. and Sotomayor, C.E. Galectins: a key intersection between glycobiology and immunology. **Brazil. J. Med. Biol. Res.** 32 (1999) 383–393.
8. Rabinovich, G.A., Baum, L.G., Tinari, N., Paganelli, R., Natoli, C., Liu, F.-T. and Iacobelli, S. Galectins and their ligands: amplifiers, silencers or tuners of the inflamatory response? **Trends Immunol.** 23 (2002) 313–320.
9. Liu, F.-T., Patterson, R.J. and Wang, J.L. Intracellular functions of galectins. **Biochim. Biophys. Acta** 1572 (2002) 263–273.
10. Perillo, N.L., Marcus, M.E. and Baum, L.G. Galectins: versatile modulators of cell adhesion, cell proliferation, and cell death. **J. Mol. Med.** 76 (1998) 402–412.
11. Wada, J. and Makino, H. Galectins, galactoside-binding mammalian lectins: clinical application of multi-functional proteins. **Acta Med. Okayama** 55 (2001) 11–17.

12. Danguy, A., Camby, I. and Kiss, R. Galectins and cancer. **Biochim. Biophys. Acta** 1572 (2002) 285–293.
13. Moutsatsos, I.K., Wade, M., Schindler, M. and Wang, J.L. Endogenous lectins from cultured cells: nuclear localization of carbohydrate-binding protein 35 in proliferating 3T3 fibroblasts. **Proc. Natl. Acad. Sci. USA** 84 (1987) 6452–6456.
14. Hirabayashi, J. and Kasai, K. The family of metazoan metal-independent beta-galactoside-binding lectins: structure, function and molecular evolution. **Glycobiology** 4 (1993) 297–304.
15. Jia, S. and Wang, J.L. Carbohydrate binding protein 35. Complementary DNA sequence reveals homology with proteins of the heterogeneous nuclear RNP. **J. Biol. Chem.** 263 (1988) 6009–6011.
16. Wang, J.L., Laing, J.G. and Anderson, R.L. Lectins in the cell nucleus. **Glycobiology** 3 (1991) 243–252.
17. Hughes, R.C. Mac-2: a versatile galactose-binding protein of mammalian tissues. **Glycobiology** 4 (1994) 5–12.
18. Birdsall, B., Feeney, J., Burdett, I.D.J., Bawumia, S., Barboni, E.A.M. and Hughes, R.C. NMR solution studies of hamster galectin-3 and electron microscopic visualization of surface-adsorbed complexes: evidence for interactions between the N- and C-terminal domains. **Biochemistry** 40 (2001) 4859–4866.
19. Huflejt, M.E., Turck, C.W., Lindstedt, R., Barondes, S.H. and Leffler, H. L-29, a soluble lactose-binding lectin, is phosphorylated on serine 6 and serine 12 in vivo and by casein kinase I. **J. Biol. Chem.** 268 (1993) 26712–26718.
20. Mazurek, N., Conklin, J., Byrd, J.C., Raz, A. and Bresalier, R.S. Phosphorylation of the β-galactoside-binding protein galectin-3 modulates binding to its ligands. **J. Biol. Chem.**. 275 (2000) 36311–36315.
21. Menon, R.P. and Hughes, R.C. Determinants in the N-terminal domains of galectin-3 for secretion by a novel pathway circumventing the endoplasmic reticulum–Golgi complex. **Eur. J. Biochem.** 264 (1999) 569–576.
22. Liao, D.I., Kapadia, G., Ahmed, H., Vasta, G.R. and Herzberg, O. Structure of S-lectin, a developmentally regulated vertebrate β-galactoside-binding protein. **Proc. Natl. Acad. Sci. USA** 91 (1994) 1428–1432.
23. Lobsanov, Y.D., Gitt, M.A., Leffler, H., Barondes, S.H. and Rini, J.M. X-ray crystal structure of the human dimeric s-Lac lectin, L-14-II, in complex with lactose at 2.9 Å resolution. **J. Biol. Chem.**. 268 (1993) 27034–27038.
24. Seetharaman, J., Kanigsberg A., Slaaby, R., Leffler, H., Barondes, S.H. and Rini, J.M. X-ray crystal structure of the human galectin-3 carbohydrate recognition domain at 2.1-Å resolution. **J. Biol. Chem.**. 273 (1998) 13047–13052.
25. Hirabayashi, J., Hashidate, T., Arata, Y., Nishi, N., Nakamura, T., Hirashima, M., Urashima, T., Oka, T., Futai, M., Muller, W.E.G., Yagi, F.

and Kasai, K. Oligosaccharide specificity of galectins: a search by frontal affinity chromatography. **Biochim. Biophys. Acta** 1572 (2002) 232–254.
26. Sato, S., Burdett, I. and Hughes, R.C. Secretion of the baby hamster kidney 30-kDa galactose-binding lectin from polarized and nonpolarized cells: a pathway independent of the endoplasmic reticulum-Golgi complex. **Exp. Cell Res.** 207 (1993) 8–18.
27. Sato, S. and Hughes, R.C. Regulation of secretion and surface expression of Mac-2, a galactoside-binding protein of macrophages. **J. Biol. Chem.**. 269 (1994) 4424–4430.
28. Nickel, W. The mystery of nonclassical protein secretion. A current view on cargo proteins and potential export routes. **Eur. J. Biochem.** 270 (2003) 2109–2119.
29. Mehul, B. and Hughes, R.C. Plasma membrane targeting, vesicular budding and release of galectin 3 from the cytoplasm of mammalian cells during secretion. **J. Cell Sci.** 110 (1997) 1169–1178.
30. Gong, H.C., Honjo, Y., Nangia-Makker, P., Hogan, V., Mazurak, N., Bresalier, R.S. and Raz, A. The NH_2 terminus of galectin-3 governs cellular compartmentalization and functions in cancer cells. **Cancer Res.** 59 (1999) 6239–6245.
31. Zlatkine, P., Mehul, B. and Magee, A.I. Retargeting of cytosolic proteins to the plasma membrane by the Lck protein tyrosine kinase dual acylation motif. **J. Cell Sci.** 110 (1997) 673–679.
32. Woo, H.-J., Shaw, L.M., Messier, J.M. and Mercurio, A.M. The major non-integrin laminin binding protein of macrophages is identical to carbohydrate binding pro-tein 35 (Mac-2). **J. Biol. Chem.** 265 (1990) 7097–7099.
33. Rosenberg, I., Cherayil, B.J., Isselbacher, K.J. and Pillai, S. Mac-2-binding glycoproteins. Putative ligands for a cytosolic β-galactoside lectin. **J. Biol. Chem.** 266 (1991) 18731–18736.
34. Koths, K., Taylor, E., Halenbeck, R., Casipit, C. and Wang, A. Cloning and characterization of a human Mac-2-binding protein, a new member of the superfamily defined by the macrophage scavenger receptor cysteine-rich domain. **J. Biol. Chem.** 268 (1993) 14245–14249.
35. Sato, S. and Hughes, R.C. Binding specificity of a baby hamster kidney lectin for H type I and II chains, polylactosamine glycans, and appropriately glycosylated forms of laminin and fibronectin. **J. Biol. Chem.** 267 (1992) 6983–6990.
36. Probstmeier, R., Montag, D. and Schachner, M. Galectin-3, a β-galactoside-binding animal lectin, binds to neural recognition molecules. **J. Neurochem.** 64 (1995) 2465–2472.
37. Ochieng, J. and Warfield, P. Galectin-3 binding potentials of mouse tumor RHS and human placental laminins. **Biochem. Biophys. Res. Commun.** 217 (1995) 402–406.

38. Dong, S. and Hughes, R.C. Macrophage surface glycoproteins binding to galectin-3 (Mac-2-antigen). **Glycoconj. J.** 14 (1997) 267–274.
39. Ochieng, J., Leite-Browning, M.L. and Warfield, P. Regulation of cellular adhesion to extracellular matrix proteins by galectin-3. **Biochem. Biophys. Res. Commun.** 246 (1998) 788–791.
40. Kuwabara, I. and Liu, F.T. Galectin-3 promotes adhesion of human neutrophils to laminin. **J. Immunol.** 156 (1996) 3939–3944.
41. Sarafian, V., Jadot, M., Foidart, J.M., Letesson, J.J., Van den Brule, F., Castronovo, V., Wattiaux, R. and Coninck, S.W. Expression of Lamp-1 and Lamp-2 and their interactions with galectin-3 in human tumor cells. **Int. J. Cancer** 75 (1998) 105–111.
42. Gonen, T., Grey, A.C., Jacobs, M.D., Donaldson, P.J. and Kistler, J. MP20, the second most abundant lens membrane protein and member of the tetraspanin superfamily, joins the list of ligands of galectin-3. **BMC Cell Biol.** 2 (2001) 17.
43. Goletz, S., Hanisch, F.-G. and Karsten, U. Novel αGalNAc containing glycans on cytokeratins are recognized in vitro by galectins with type II carbohydrate recognition domains. **J. Cell Sci.** 110 (1997) 1585–1596.
44. Sève, A.-P., Felin, M., Doyennette-Moyne, M.-A., Sahraoui, T., Aubery, M. and Hubert, J. Evidence for a lactose-mediated association between two nuclear carbohydrate-binding proteins. **Glycobiology** 3 (1993) 23–30.
45. Menon, R.P., Strom, M. and Hughes, R.C. Interaction of a novel cysteine and histidine-rich cytoplasmic protein with galectin-3 in a carbohydrate-independent manner. **FEBS Lett.** 470 (2000) 227–231.
46. Park, J.W., Voss, P.G., Grabski, S., Wang, J.L. and Patterson, R.J. Association of galectin-1 and galectin-3 with Gemin4 in complexes containing the SMN protein. **Nucl. Acids Res.** 27 (2001) 3595–3602.
47. Akahani, S., Nangia-Makker, P., Inohara, H., Kim, H.-R.C. and Raz, A. Galectin-3: a novel antiapoptotic molecule with a functional BH1 (NWGR) domain of Bcl-2 family. **Cancer Res.** 57 (1997) 5272–5276.
48. Rousseau, C., Muriel, M.-P., Musset, M., Botti, J. and Sève, A.-P. Glycosylated nuclear lectin CBP70 also associated with endoplasmic reticulum and the Golgi apparatus: does the “classic pathway” of glycosylation also apply to nuclear glycoproteins? **J. Cell. Biochem.** 78 (2000) 638–649.
49. Bawumia S., Barboni, E.A.M., Menon, R.P. and Hughes, R.C. Specificity of interactions of galectin-3 with Chrp, a cysteine- and histidine-rich cytoplasmic protein. **Biochimie** 85 (2003) 189–194.
50. Vito, P., Pellegrini, L., Guiet, C. and D’Adamio, L. Cloning of AIP1, a novel protein that associates with the apoptosis-linked gene ALG-2 in a Ca^{2+}-dependent reaction. **J. Biol. Chem.** 274 (1999) 1533–1540.
51. Ohannesian, D.W., Lotan, D., Thoman, P., Jessup, J.M., Fukuda, M., Gabius, H.J. and Lotan, R. Carcinoembryonic antigen and other

glycoconjugates act as ligands for galectin-3 in human colon carcinoma cells. **Cancer Res.** 55 (1995) 2191–2199.
52. Yang, R.-Y., Hsu, D.K. and Liu, F.-T. Expression of galectin-3 modulates T-cell growth and apoptosis. **Proc. Natl. Acad. Sci. USA** 93 (1996) 6737–6742.
53. Vlassara, H., Li, Y.M., Imani, F., Wojciechowicz, D., Yang, Z., Liu, F.T., and Cerami, A. Identification of galectin 3 as a high affinity binding protein for advanced glycation end products (AGE): a new member of the AGE-receptor complex. **Mol. Med.** 1 (1995) 634–646.
54. Pricci, F., Leto, G., Amadio, L., Iacobini, C., Romeo, G., Cordone, S., Gradini, R., Barsotti, P., Liu, F.T., Di Mario, U., and Pugliese, G. Role of galectin 3 as a receptor for advanced glycosylation end products. **Kidney Int.** 58 (2000) S31–S39.
55. Zhu, W., Sano, H., Nagai, R., Fukuhara, K., Miyazaki, A., and Horiuchi, S. The role of galectin-3 in endocytosis of advanced glycation end products and modified low density lipoproteins. **Biochem. Biophys. Res. Commun.** 280 (2001) 1183–1188.
56. Matarrese, P., Tinari, N., Semeraro, M.L., Natoli, C., Iacobelli, S. and Malorni, W. Galectin-3 overexpression protects from cell damage and death by influencing mitochondrial homeostasis. **FEBS Lett.** 473 (2000) 311–315.
57. Matarrese, P., Fusco, O., Tinari, N., Natoli, C., Liu F.-T., Semeraro, M.L., Malorni, W. and Iacobelli, S. Galectin-3 overexpression protects from apoptosis by improving cell adhesion properties. **Int. J. Cancer** 85 (2000) 545–554.
58. Moon, B.-K., Lee, Y.J., Battle, P., Jessup, J.M., Raz, A. and Kim, H.-R.C. Galectin-3 protects human breast carcinoma cells against nitric oxide-induced apoptosis. Implication of galectin-3 function during metastasis. **Am. J. Pathol.** 159 (2001) 1055–1060.
59. Hanada M., Aimé-Sempé C., Sato, T. and Reed, J.C. Structure-function analysis of Bcl-2 protein. **J. Biol. Chem.**. 270 (1995) 11962–11969.
60. Nagata, S. Apoptosis. **Fibrinolysis & Proteolysis** 14 (2000) 82–86.
61. Zörnig, M., Hueber, A.-O., Baum, W. and Evan, G. Apoptosis regulators and their role in tumorigenesis. **Biochim. Biophys. Acta** 1551 (2001) F1–F37.
62. Yu, F., Finley, R.L., Jr., Raz, A. and Kim, H.-R.C. Galectin-3 translocates to the perinuclear membranes and inhibits cytochrome *c* release from the mitochondria. A role for synexin in galectin-3 translocation. **J. Biol. Chem.**. 277 (2002) 15819-15827.
63. Kim, H.-R.C., Lin, H.-M., Biliran, H. and Raz, A. Cell cycle arrest and inhibition of anoikis by galectin-3 in human breast epithelial cells. **Cancer Res.** 59 (1999) 4148-4154.

64. Lin, H.-M., Moon, B.-K., Yu, F. and Kim, H.-R.C. Galectin-3 mediates genistein--induced G_2/M arrest and inhibits apoptosis. **Carcinogenesis** 21 (2000) 1941–1945.
65. Wang, L., Inohara, H., Pienta, K.J. and Raz, A. Galectin-3 is a nuclear matrix protein which binds RNA. **Biochem. Biophys. Res. Commun.** 217 (1995) 292–303.
66. Dagher, S.F., Wang, J.L. and Patterson, R.J. Identification of galectin-3 as a factor in pre-mRNA splicing. **Proc. Natl. Acad. Sci. USA** 92 (1995) 1213–1217.
67. Lin, H.-M., Pestell, R.G., Raz, A. and Kim, H.-R.C. Galectin-3 enhances cyclin D_1 promoter activity through SP1 and a cAMP-responsive element in human breast epithelial cells. **Oncogene** 21 (2002) 8001–8010.
68. Yoshii, T., Fukumori, T., Honjo, Y., Inohara, H., Kim, H.-R.C. and Raz, A. Galectin-3 phosphorylation is required for its anti-apoptotic function and cell cycle arrest. **J. Biol. Chem.** 277 (2002) 6852–6857.
69. Xu, X.-C., El-Naggar, A.K. and Lotan, R. Differential expression of galectin-1 and galectin-3 in thyroid tumors. Potential diagnostic implications. **Am. J. Pathol.** 147 (1995) 815–822.
70. Fernández, P.L., Merino, M.J., Gómez M., Campo, E., Medina, T., Castronovo, V., Sanjuán, X., Cardesa, A., Liu, F.-T. and Sobel, M.E. Galectin-3 and laminin expression in neoplastic and non-neoplastic thyroid tissue. **J. Pathol.** 181 (1997) 80–86.
71. Orlandi, F., Saggiorato, E., Pivano, G., Puligheddu, B., Termine, A., Cappia, S., De Giuli, P. and Angeli, A. Galectin-3 is a presurgical marker of human thyroid carcinoma. **Cancer Res.** 58 (1998) 3015–3020.
72. Inohara, H., Honjo, Y., Yoshii, T., Akahani, S., Yoshida, J., Hattori, K., Okamoto, S., Sawada, T., Raz, A. and Kubo, T. Expression of galectin-3 in fine-needle aspirates as a diagnostic marker differentiating benign from malignant thyroid neoplasms. **Cancer** 85 (1999) 2475–2484.
73. Cvejic, D., Savin, S., Golubovic, S., Paunovic, I., Tatic, S. and Havelka, M. Galectin-3 and carcinoembryonic antigen expression in medullary thyroid carcinoma: possible relation to tumour progression. **Histopathology** 37 (2000) 530–535.
74. Kawachi, K., Matsushita, Y., Yonezawa, S., Nakano, S., Shirao, K., Natsugoe, S., Sueyoshi, K., Aikou, T. and Sato, E. Galectin-3 expression in various thyroid neoplasms and its possible role in metastasis formation. **Human Pathol.** 31 (2000) 428–433.
75. Yoshii, T., Inohara, H., Takenaka, Y., Honjo, Y., Akahani, S., Nomura, T., Raz, A. and Kubo, T. Galectin-3 maintains the transformed phenotype of thyroid papillary carcinoma cells. **Int. J. Oncol.** 18 (2001) 787–792.
76. Saggiorato, E., Cappia, S., De Gliuli, P., Mussa, A., Pancani, G., Caraci, P., Angeli, A. and Orlandi, F. Galectin-3 as a presurgical immuno-cytodiagnostic marker of minimally invasive follicular thyroid carcinoma. **J. Clin. Endocrinol. Metab.** 86 (2001) 5152–5158.

77. Coli, A., Bigotti, G., Zuchetti, F., Negro, F. and Massi, G. Galectin-3, a marker of well-differentiated thyroid carcinoma, is expressed in thyroid nodules with cytological atypia. **Histopathology** 40 (2002) 80–87.
78. Faggiano, A., Talbot, M., Lacroix, L., Bidart, J.M., Baudin, E., Schlumberger, M. and Caillou, B. Differential expression of galectin-3 in medullary thyroid carcinoma and C-cell hyperplasia. **Clin. Endocrinol.** 57 (2002) 813–819.
79. Herrmann, M.E., LiVolsi, V.A., Pasha, T.L., Roberts, S.A., Wojcik, E.M. and Baloch, Z.W. Immunohistochemical expression of galectin-3 in benign and malignant thyroid lesions. **Arch. Pathol. Lab. Med.** 126 (2002) 710–713.
80. Takenaka, Y., Inohara, H., Yoshii, T., Oshima, K., Nakahara, S., Akahani, S., Honjo, Y., Yamamoto, Y., Raz, A. and Kubo, T. Malignant transformation of thyroid follicular cells by galectin-3. **Cancer Lett.** 195 (2003) 111–119.
81. Berberat, P.O., Friess, H., Wang, L., Zhu, Z., Bley, T., Frigeri, L., Zimmermann, A., and Büchler, M.W. Comparative analysis of galectins in primary tumors and tumor metastasis in human pancreatic cancer. **J. Histochem. Cytochem.** 49 (2001) 539–549.
82. Hsu, D.K., Dowling, C.A., Jeng, K.-C.G., Chen, J.-T., Yang, R.-Y. and Liu, F.-T. Galectin-3 expression is induced in cirrhotic liver and hepatocellular carcinoma. **Int. J. Cancer** 81 (1999) 519–526.
83. Castronovo, V., Campo, E., van den Brûle, F.A., Claysmith, A.P., Cioce, V., Liu, F.-T., Fernandez, P.L. and Sobel, M.E. Inverse modulation of steady-state messenger RNA levels of two non-integrin laminin-binding proteins in human colon carcinoma. **J. Natl. Cancer Inst.** 84 (1992) 1161–1169.
84. Lotz, M.M., Andrews, C.W. Jr., Korzelius, C.A., Lee, E.C., Steele, G.D. Jr., Clarke, A. and Mercurio, A.M. Decreased expression of Mac-2 (carbohydrate binding protein 35) and loss of its nuclear localization are associated with the neoplastic progression of colon carcinoma. **Proc. Natl. Acad. Sci. USA** 90 (1993) 3466–3470.
85. Irimura, T., Matsushita, Y., Sutton, R.C., Carralero, D., Ohannesian, D.W., Cleary, K.R., Ota, D.M., Nicolson, G.L. and Lotan, R. Increased content of a endogenous lactose-binding lectin in human colorectal carcinoma progressed to metastatic stages. **Cancer Res.** 51 (1991) 387–393.
86. Schoeppner, H.L., Raz, A., Ho, S.B. and Bresalier, R.S. Expression of an endogenous galactose-binding lectin correlates with neoplastic progression in the colon. **Cancer** 75 (1995) 2818–2826.
87. Sanjuán, X., Fernández, P.L., Castells, A., Castronovo, V., van der Brule, F., Liu, F.-T., Cardesa, A. and Campo, E. Differential expression of galectin 3 and galectin 1 in colorectal cancer progression. **Gastroenterology** 113 (1997) 1906–1915.

88. Nagy, N., Legendre, H., Engels, O., André, S., Kaltner, H., Wasano, K., Zick, Y., Pector, J.-C., Decaestecker, C., Gabius, H.-J., Salmon, I. and Kiss, R. Refined prognostic evaluation in colon carcinoma using immunohistochemical galectin finger-printing. **Cancer** 97 (2003) 1849–1858.
89. Bresalier, R.S., Mazurek, N., Sternberg, L.R., Byrd, J.C., Yunker, C.K., Nangia--Makker, P. and Raz, A. Metastasis of human colon cancer is altered by modifying expression of the β-galactoside-binding protein galectin 3. **Gastroenterology** 115 (1998) 287–296.
90. Dudas, S.P., Yunker, C.K., Sternberg, L.R., Byrd, J.C. and Bresalier, R.S. Expression of human intestinal mucin is modulated by the β-galactoside binding protein galectin-3 in colon cancer. **Gastroenterology** 123 (2002) 817–826.
91. Castronovo, V., Van Den Brûle, F.A., Jackers, P., Clusse, N., Liu, F.T., Gillet, C. and Sobel, M.E. Decreased expression of galectin-3 is associated with progression of human breast cancer. **J. Pathol.** 179 (1996) 43–48.
92. Idikio, H. Galectin-3 expression in human breast carcinoma: correlation with cancer histologic grade. **Int. J. Oncol.** 12 (1998) 1287–1290.
93. Honjo, Y., Nangia-Makker, P., Inohara, H. and Raz, A. Down-regulation of galectin-3 suppresses tumorigenecity of human breast carcinoma cells. **Clin. Cancer Res.** 7 (2001) 661–668.
94. Song, Y.K., Billiar, T.R. and Lee, Y.J. Role of galectin-3 in breast cancer metastasis. Involvement of nitric oxide. **Am. J. Pathol.** 160 (2002) 1069–1075.
95. Ellerhorst, J., Troncoso, P., Xu, X.C., Lee, J. and Lotan, R. Galectin-1 and galectin-3 expression in human prostate tissue and prostate cancer. **Urol. Res.** 27 (1999) 362–367.
96. Pacis, R.A., Pilat, M.J., Pienta, K.J., Wojno, K., Raz, A., Hogan, V. and Cooper, C.R. Decreased galectin-3 expression in prostate cancer. **Prostate** 44 (2000) 118–123.
97. Van den Brûle, F.A., Waltregny, D., Liu, F.-T. and Castronovo, V. Alteration of the cytoplasmic/nuclear expression pattern of galectin-3 correlates with prostate carcinoma progression. **Int. J. Cancer** 89 (2000) 361–367.
98. Choufani, G., Nagy, N., Saussez, S., Marchant, H., Bisschop, P., Burchert, M., Danguy, A., Louryan, S., Salmon, I., Gabius, H.-J., Kiss, R. and Hassid, S. The levels of expression of galectin-1, galectin-3, and the Thomsen-Friedenreich antigen and their binding sites decrease as clinical aggressiveness increases in head and neck cancers. **Cancer** 86 (1999) 2353–2363.
99. Delorge, S., Saussez, S., Pelc, P., Devroede, B., Marchant, H., Burchert, M., Zeng, F.Y., Danguy, A., Salmon, I., Gabius, H.J., Kiss, R. and Hassid, S. Correlation of galectin-3/galectin-3-binding sites with low differentiation

status in head and neck squamous cell carcinomas. **Otolaryngol. Head Neck Surg.** 122 (2000) 834–841.

100. Lefranc, F., Chevalier, C., Vinchon, M., Dhellemmes, P., Schüring, M.-P., Kaltner, H., Brotchi, J., Ruchoux, M.-M., Gabius, H.-J., Salmon, I. and Kiss, R. Characterization of the levels of expression of retinoic acid receptors, galectin-3, macrophage migration inhibiting factor, and p53 in 51 adamantinomatous craniopharyngiomas. **J. Neurosurg.** 98 (2003) 145–153.
101. Sheikholeslam-Zadeh, R., Decaestecker, C., Delbrouck, C., Danguy, A., Salmon, I., Zick, Y., Kaltner, H., Hassid, S., Gabius, H.-J., Kiss, R. and Choufani, G. The levels of expression of galectin-3, but not of galectin-1 and galectin-8, correlate with apoptosis in human cholesteatomas. **Laryngoscope** 111 (2001) 1042–1047.
102. Honjo, Y., Inohara, H., Akahani, S., Yoshii, T., Takenaka, Y., Yoshida, J., Hattori, K., Tomiyama, Y., Raz, A. and Kubo, T. Expression of cytoplasmic galectin-3 as a prognostic marker in tongue carcinoma. **Clin. Cancer Res.** 6 (2000) 4635–4640.
103. Bresalier, R.S., Yan, P.-S., Byrd, J.C., Lotan, R. and Raz, A. Expression of the endogenous galactose-binding protein galectin-3 correlates with the malignant potential of tumors in the central nervous system. **Cancer** 80 (1997) 776–787.
104. Gordower, L., Decaestecker, C., Kacem, Y., Lemmers, A., Gusman, J., Burchert, M., Danguy, A., Gabius, H., Salmon, I., Kiss, R. and Camby, I. Galectin-3 and galectin-3-binding site expression in human adult astrocytic tumours and related angiogenesis. **Neuropathol. Appl. Neurobiol.** 25 (1999) 319–330.
105. Camby, I., Belot, N., Rorive, S., Lefranc, F., Maurage, C.-A., Lahm, H., Kaltner, H., Hadari, Y., Ruchoux, M.-M., Brotchi, J., Zick, Y., Salmon, I., Gabius, H.-J. and Kiss, R. Galectins are differentially expressed in supratentorial pilocytic astrocytomas, astrocytomas, anaplastic astrocytomas and glioblastomas, and significantly modulate tumor astrocytes migration. **Brain Pathol.** 11 (2001) 12–26.