

Review

Galectin-3: An open-ended story

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Abstract

Galectins, an ancient lectin family, are characterized by specific binding of β -galactosides through evolutionary conserved sequence elements of carbohydrate-recognition domain (CRD). A structurally unique member of the family is galectin-3; in addition to the CRD it contains a proline- and glycine-rich N-terminal domain (ND) through which is able to form oligomers. Galectin-3 is widely spread among different types of cells and tissues, found intracellularly in nucleus and cytoplasm or secreted via non-classical pathway outside of cell, thus being found on the cell surface or in the extracellular space. Through specific interactions with a variety of intra- and extracellular proteins galectin-3 affects numerous biological processes and seems to be involved in different physiological and pathophysiological conditions, such as development, immune reactions, and neoplastic transformation and metastasis. The review attempts to summarize the existing information on structural, biochemical and intriguing functional properties of galectin-3.

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Within the busy protein world lectins are a unique group of proteins assigned to provide an interpreter service for the biological information encoded within specific oligosaccharide structures of glycoconjugates. In a way, they bridge proteomics and glycomics. Lectins are classified into families, among which galectins are an ancient and particularly interesting one. Galectins are defined by evolutionary conserved amino acid sequences and by recognition of β -galactoside structures [1]. Members of galectin family were found in sponges, fungi, nematodes, insects, vertebrates including mammals, and even viral galectins have been identified (reviewed in [2]). Their homologues are also present in plants [3], but not in yeasts. Thus far, 14 mammalian galectins have been identified, all containing a conserved carbohydrate-recognition-binding domain (CRD) of about 130 amino acids. Based on the number and on the organization of CRDs, members of galectin family have been classified into three subtypes: the prototype group, the chimera group and the tandem repeat group [4]. The

members of the prototype group (galectin-1, -2, -5, -7, -10, -11, -13, and -14) contain one carbohydrate-recognition domain. Galectin-3, the only vertebrate chimera type galectin, also contains one CRD connected to an unusual long N-terminal proline- and glycine-rich domain. Galectin-4, -6, -8, -9, and -12, the members of the tandem repeat group are built of a single polypeptide chain that forms two distinct but homologous CRDs, separated by an unconserved linker sequence of up to 70 amino acids. Thus, the two-CRD type galectins can bind two individual carbohydrate epitopes. In addition, some one-CRD containing galectins are able to form dimers or oligomers depending on specific conditions (concentration or presence of ligands); this enables bivalent or multivalent binding of carbohydrate ligands which is crucial for some of their biological functions. Although the presence of galactose is essential for all galectins' binding, the affinity for the monosaccharide ligand is rather weak, with K_d values in mM range. The binding affinity of galectins increases if galactose is attached to other saccharides, e.g., N-acetylglucosamine forming N-acetyllactosamine [5].

The vertebrate galectins were found in the cytoplasm and the nucleus, on the cell surface, and in the extracellular space. The presence of galectins outside of the cell is a consequence of

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secretion via non-classical pathway, since they lack a signal sequence for insertion into the endoplasmic reticulum [6], with the possible exceptions of a sponge galectin [7] and several other invertebrate gene products [2].

Galectins were detected in numerous cell and tissue types, and various functions were ascribed to them. Multiplicity and diversity of their functions make galectins intriguing enough to become emerging research attractors, not only in glycobiology, but also in medicine and pharmacy.

The most studied member of the galectin family is galectin-3, a ubiquitously present protein with a variety of biological roles. This review attempts to summarize its structural, biochemical and functional properties discussing an open-ended story.

2. Structural and biochemical characteristics of galectin-3

Galectin-3, a 29- to 35-kDa protein, was initially identified as Mac-2, a 32-kDa cell surface antigen expressed on murine thioglycollate-elicited peritoneal macrophages [8]. Later it was also described as CBP-35, a 35-kDa carbohydrate-binding protein in mouse fibroblasts [9]; ϵ BP, an ϵ -binding protein from rat basophilic leukemia cells [10]; RL-29, a 29-kDa lectin in rat lung tissue [11] and HL-29, in human lung tissue [12]; L-34, a 34-kDa lectin in oncogene-transfected rat embryonal fibroblasts [13], and LBP, a non-integrin laminin binding protein in macrophages [14]. The analysis of amino acid and gene sequences of these proteins showed high homology between proteins isolated from different species [14–22]. In accordance with nomenclature introduced in 1994, this protein was designated as galectin-3 [1].

The structure of galectin-3 seems to be unique among all vertebrate galectins [23]; its single polypeptide chain forms two structurally distinct domains, atypical N-terminal domain (ND) and C-terminal carbohydrate-recognition domain. Many studies of physico-chemical characteristics of galectin-3 suggested not only profound structural, but also functional differences of these two domains [24–26].

2.1. The N-terminal domain

The N-terminal domain of galectin-3 is composed of 110–130 amino acids, depending on species. This relatively flexible structure contains multiple homologues repeats (7–14), each of which includes a consensus sequence Pro–Gly–Ala–Tyr–Pro–Gly, followed by three additional amino acids. The ND is highly conserved among galectin-3 molecules isolated from different species. In addition, the amino acid sequence is approximately 25% homologous with some heterogeneous nuclear ribonucleoprotein (hnRNP) complexes what corresponds to the homology found among the core hnRNP proteins themselves [27]. It has been reported that the ND has 33.5% identity with collagen α 1 (II) chain of bovine cartilage [28], so the ND is also designated as a collagen-like N-terminal domain.

Although the ND was shown to lack carbohydrate-binding activity, it is essential for full biological activity of galectin-3 [29]. Furthermore, more recent results obtained by molecular modeling and mutagenesis analysis revealed that the ND,

through Tyr¹⁰² and adjacent residues, participates together with the CRD in oligosaccharide binding [30]. The ND is also responsible for multimer forming and shows positive cooperativity in lectin binding to immobilized ligand clusters [31]. This property seems to be biologically regulated, because the ND is susceptible to selective proteolysis by certain matrix metalloproteinase, MMP-2 and MMP-9 [32]. The cleavage at the position Ala⁶²–Tyr⁶³ of the recombinant human galectin-3 increases the affinity of the CRD (preserved in 22 kDa fragment) to the carbohydrate ligands, but reduces self-association of galectin-3, abrogating in that way biological properties dependant on multimerization. Thus, for example, proteolytically cleaved galectin-3 displays approximately 20-fold higher binding affinity for human umbilical vein endothelial cells as compared to the full-length protein [33]. The ND has been also implicated in secretion of galectin-3 outside of cells [34]. The initial 12 amino acid N-terminal peptide sequence preceding the proline/glycin-rich repetitive domain, also called small N-terminal domain, is highly conserved in all mammalian galectin-3. At least two functional characteristics were ascribed to this N-terminal portion of galectin-3; deletion of these first 11 amino acids (following the first methionine) blocks secretion of galectin-3 [35], while mutation of the conserved Ser⁶ affects galectin-3 anti-apoptotic signaling activity [36] (see below).

2.2. The carbohydrate-recognition domain (CRD)

The C-terminal domain of galectin-3, composed of about 130 amino acids forming a globular structure, accommodates whole carbohydrate-binding site, thus being responsible for lectin activity of galectin-3 [24,37]. These findings were additionally confirmed by X-ray crystal structure analysis that revealed the folding pattern (two anti-parallel β -sheets, composed of five and six β -strands) typical for carbohydrate-recognition domain of other galectins [29]. On the contrary to the previous reports [25], more recent results obtained by nuclear magnetic resonance analysis suggested possible interactions between portions of the ND and the CRD [38].

Within the carbohydrate-recognition domain particularly interesting amino acid sequence is NWGR; this motif is highly conserved within the BH1 domain of the Bcl-2 family proteins, and it was shown to be responsible for the anti-apoptotic activity of both Bcl-2 and galectin-3 [39] (see below). The NWGR motif is also involved in self-association of galectin-3 molecules through the CRDs in the absence of saccharide ligands [40]. The replacement of tryptophan with leucine (W181L) within the NWGR motif abolishes homodimerization through the CRDs of galectin-3. However, this mutant can still bind wild-type galectin-3 through the interactions of N-terminal domains. The CRD is also involved in carbohydrate-dependent homophilic interactions of galectin-3 [41]. Single cysteine residue suited near NWGR motif (Cys¹⁸⁶) was shown to be required for dimerization of murine galectin-3, which in that form binds laminin with higher affinity than monomeric form [42]. The CRD, comparing to the intact galectin-3, exhibits stronger binding affinity for advanced glycation end-products (AGE),

what suggests that the CRD may also contain the principal AGE-binding site which is sterically hindered by the ND in the full-length galectin-3 [43].

2.3. The sugar-binding specificity and multivalent properties of galectin-3

The studies on carbohydrate-binding activity and specificity of galectin-3 described N-acetyllactosamine (LacNAc, Gal β 1,4(3)GlcNAc) as its preferential ligand [25,44]. Furthermore, galectin-3 was shown to possess an extended binding site which can accommodate longer oligosaccharides such as polylactosaminoglycans [45], what was additionally confirmed by X-ray crystal analysis of human galectin-3 CRD [29]. These structural studies together with mutagenesis data implicated that Arg139 of hamster/Arg144 of human galectin-3, absent in other galectins, are involved in the binding of sugar units linked to O-3 of a terminal β -linked galactose residue in structures such as NeuNAc- α 2,3-Gal- β 1,4-Glc or GalNAc- α 1,3-[Fuc- α 1,2]-Gal- β 1,4-Glc [46]. For detailed information on oligosaccharide specificity of galectin-3, please see the review reported by Hirabayashi et al. [47].

The interaction of galectin-3 or its CRD with carbohydrate ligands is accompanied by a conformational change [25] and rearrangement of the backbone loops near the binding site [48]. In addition, the phosphorylation at the position Ser⁶ of galectin-3 strongly affects its sugar binding affinity, so it was proposed to be an “on/off” switch of its downstream biological effects [49].

As it was previously mentioned, galectin-3 exhibits bi-/multivalent binding properties, although it possesses only one carbohydrate-recognition domain [42,45]. Both, carbohydrate-recognition domain and N-terminal domain were shown to be involved in formation of galectin-3 multimers. The recent demonstration that galectin-3 molecules assemble in pentamer formation in the presence of multivalent ligands, in process mediated through their N-terminal domains [50], further supported the hypothesis that the ND is responsible for galectin-3 multimerization. However, these experiments were performed in vitro conditions therefore additional experiments are required to elucidate physico-chemical properties of galectin-3 multimer formation, especially in vivo.

There are numerous biological ligands of galectin-3 which are structurally and functionally very diverse (Figs. 1 and 2). With some of them galectin-3 interacts via terminal N-

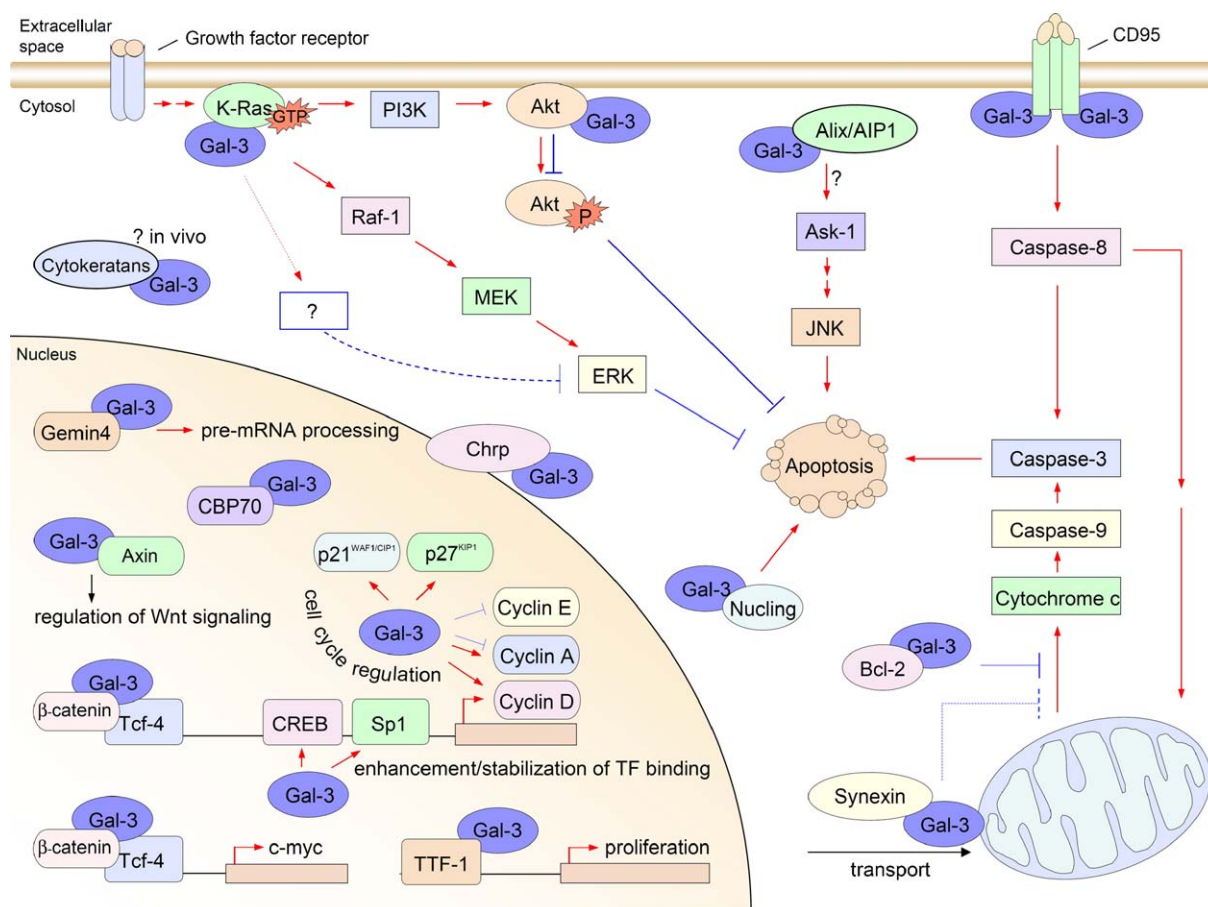


Fig. 1. The intracellular functions of galectin-3. Red arrows indicate positive effects, blue lines indicate negative effects. Akt—the serine/threonine kinase Akt, Ask-1—apoptosis signal-regulating kinase 1, CBP70—carbohydrate binding protein 70, Chrp—cysteine- and histidine-rich protein, CREB—cAMP-response element-binding protein, ERK—extracellular signal-regulated kinase, Gal-3—galectin-3, GTP—guanosine triphosphate, JNK—c-Jun NH₂-terminal kinase, MEK—mitogen-activated protein/ERK kinase, P—phosphate, PI3K—phosphatidylinositol 3-kinase, Raf-1—the serine/threonine kinase Raf-1, Tcf-4—T cell factor 4, TF—transcription factor, TTF-1—thyroid-specific transcription factor.

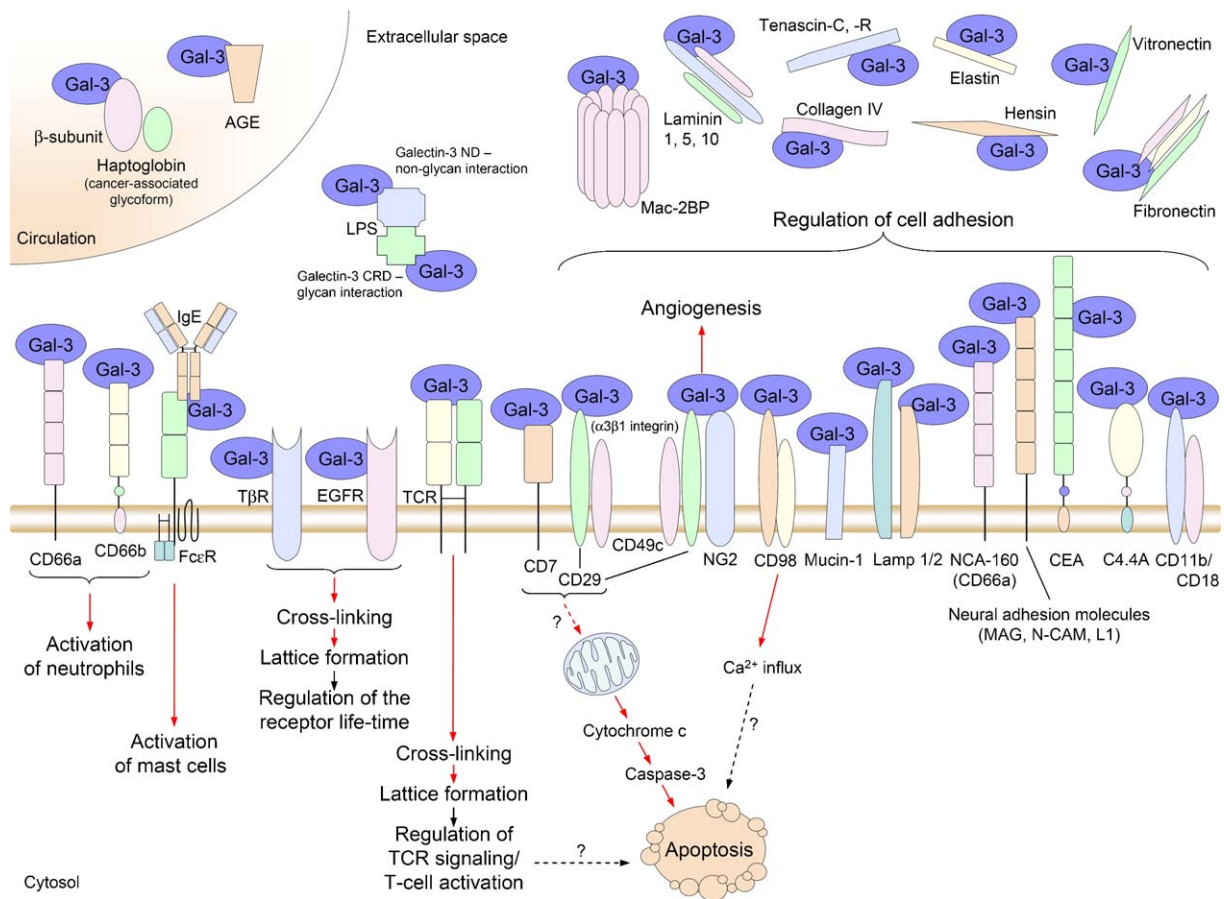


Fig. 2. The extracellular functions of galectin-3. Red arrows indicate positive effects. AGE—advanced glycation end products, C4.4A—the GPI-anchored glycoprotein C4.4A, CEA—carcinoembryonic antigen, CRD—carbohydrate-recognition domain, EGFR—epidermal growth factor receptor, FcεR—Fce receptor, Gal-3—galectin-3, IgE—immunoglobulin E, L1—neural adhesion molecule L1, Lamp 1/2—lysosome associated membrane protein 1/2, LPS—lipopolysaccharide, Mac-2BP—Mac-2 binding protein, MAG—myelin associated glycoprotein, N-CAM—neural cell adhesion molecule, NCA-160—non-specific cross-reacting antigen 160, ND—N-terminal domain, NG2—the transmembrane chondroitin sulfate proteoglycan NG2, TβR—transforming growth factor β receptor, TCR—T cell receptor.

acetyllactosamine (LacNAc) residues present in their glycan parts, and these interactions can be inhibited by lactose. However, not all LacNAc-bearing glycoproteins proved to be galectin-3 counterparts, possibly because the binding affinity is very much lower in comparison with other galectins, as a consequence of sterical interference by other parts of glycan chain or because of specific protein folding. On the other hand, the specificity of galectin-3 binding might be enhanced by the presence of a particular peptide sequence in the surroundings of LacNAc residue. It is also possible that some in vitro ligands of galectin-3 are not biologically relevant counterparts because they are not expressed in cell and tissues where galectin-3 is present, or vice versa. Furthermore, the expression of galectin-3 and its counterpart may not be time-coordinated. Taken together, not only sugar-binding affinity of galectin-3 and its phosphorylation status determine the specificity of binding to a particular biological counterpart, but also the time- and space-coordination of their expression, as well as the structure of both, glycan and protein component of the counterpart.

The expression of specific glycan structures strongly depends on activity of glycosyltransferases, especially β-N-acetylgluco-

saminyl transferases and galactosyltransferases. One of them, β1,6-N-acetylglucosaminyl transferase (Mgat5, GnT-V) promotes addition of N-acetyllactosamine on N-glycans, thus creating a preferred ligands of galectin-3. Demetriou et al. [51] showed that T cells from Mgat5-deficient mice have lower activation thresholds and enhanced T cell receptor (TCR) clustering. In wild-type mice, galectin-3 is associated with the TCR complex at the T cell surface; pre-treatment of these cells with lactose to compete galectin-3 binding results in hyperactivation of the cells, also observed in Mgat5-deficient mice. These results suggest that galectin-3 participates in the formation of galectin-3-TCR lattices, which potentially restrict the lateral movement of TCR and raise the threshold for ligand-dependent receptor clustering and signal transduction, thus preventing uncontrolled activation of T cells. As well, galectin-3 cross-links Mgat5-modified N-glycans on epidermal growth factor (EGF) receptors and transforming growth factor β (TGFβ) receptors and delays their removal by constitutive endocytosis [52].

Besides carbohydrate-bearing counterparts, galectin-3 interacts with several unglycosylated molecules through protein–protein interactions [53–59] (see below).

2.4. The phosphorylation of galectin-3

In addition to the aforementioned phosphorylation of galectin-3 at the position Ser⁶, this protein can undergo phosphorylation at the position Ser¹² [60]. However, the majority of phosphate is present at Ser⁶, while less than 10% at Ser¹², as it was shown by mass spectroscopic analysis of canine galectin-3 [61]. Casein kinase I and casein kinase II were shown to effectively phosphorylate Ser⁶ in vitro, but the enzyme responsible for in vivo phosphorylation of galectin-3 at position Ser⁶ has not yet been identified.

The presence of both phosphorylated (pI 8.2) and unphosphorylated form (pI 8.7) of galectin-3 has been reported for the first time in murine 3T3 fibroblasts; phosphorylated galectin-3 (pI 8.2) was found in both the cytoplasm and the nucleus, whereas unphosphorylated form (pI 8.7) was found exclusively in the cytoplasm [60]. These findings suggested requirement of phosphorylation for transport of galectin-3 into the nucleus, but mutagenesis studies revealed that Ser⁶ is not essential for nuclear localization [35]. In vitro, human galectin-3 can be phosphorylated only at Ser⁶ by casein kinase I. The phosphorylation considerably reduces its binding ability to the ligands, but the binding ability can be fully restored by the dephosphorylation of galectin-3 with protein phosphatase type 1 [49]. Thus, the phosphorylation at position Ser⁶ was suggested to be a regulatory modification of multivalent binding activity of galectin-3.

Recent findings revealed the presence of glycogen synthase kinase-3 β (GSK-3 β) consensus motif (S⁹²XXXXS⁹⁶) in galectin-3 sequence and showed that GSK-3 β phosphorylates galectin-3 in a time- and dose-dependent manner [59]. However, biological consequences and precise mechanism of the effects of GSK-3 β galectin-3 phosphorylation on counter-part molecules is yet to be elucidated.

Besides the phosphorylation at serine residues, it was suggested that galectin-3 can undergo phosphorylation at a tyrosine or even tyrosines, during a distinct period of repair in hepatocytes injured by administration of CCl₄ [62].

In addition to the role in the regulation of carbohydrate-binding activity of galectin-3, phosphorylation was shown to affect some other biological effects of this protein that will be discussed later.

3. Tissue distribution of galectin-3

In adults, galectin-3 is ubiquitously expressed. However, during mouse embryogenesis its expression is tissue- and time-dependent. The onset of galectin-3 expression occurs during fourth day of gestation when the protein is detected in the cells of trophectoderm of blastocyst [63]. Between 8.5 and 11.5 days of gestation galectin-3 is exclusively expressed in the notochord cells [64]. In later stages of mouse development, galectin-3 localization is observed in the cartilage of vertebrae, ribs and facial bones, the suprabasal layer of epidermis, the endodermal lining of the bladder, larynx and oesophagus. The punctate expression observed in some organs such as liver and lungs, as well as mineralizing part of the bones, was suggested to be

associated with macrophages and/or related cell types such as osteoclasts. The expression of galectin-3 during first trimester of human embryogenesis is restricted to epithelia, such as the skin, epithelial lining of the digestive and respiratory tract, urothelium and excretory tubes of the kidney, in the myocardial cells, in the peripheral and preossifying hypertrophic chondrocytes, as well as in notochord and in the liver [65].

Although it was found in many normal tissues, galectin-3 expression in adults, similarly to its expression during embryogenesis, is mainly related to the epithelial cells and myeloid/amoeboic cells. Galectin-3 was found in small intestinal epithelial cells [66], in colonic epithelia [67], in corneal [68–70] and conjunctival epithelia [69], in restricted area of olfactory epithelium [71], in the epithelial cells of kidney [72], lung [73], thymus [74], breast [75], and prostate [76]. It was also detected in ductal cells of salivary glands [77], pancreas [78,79], kidney [80], and eye [81] and in intrahepatic bile ducts [82]. As well, galectin-3 was shown to be expressed in uterine epithelia of pregnant animals immediately after implantation, but not in non-pregnant animals, or during the pre-implantation stages of pregnancy [83]. It was also found in fibroblasts [84], chondrocytes and osteoblasts [85,86], osteoclasts [87], keratinocytes [88,89], Schwann cells [90] and gastric mucosa [91], as well as in the endothelial cells from various tissues and organs [92]. In addition, there are numerous data on galectin-3 expression in the cells involved in immune response, such as neutrophils [93], eosinophils [94], basophils and mast cells [95,96], Langerhans cells [88,97], dendritic cells [66,98], as well as monocytes [99] and macrophages from different tissues [66,73,100,101]. In some other cell types, such as lymphocytes, galectin-3 is not normally expressed, yet its expression can be induced by various stimuli (see below).

Galectin-3 is also expressed in a variety of tumors, and the intensity of the expression depends on tumor progression, invasiveness and metastatic potential (reviewed in [102,103]).

4. *LGALS3* gene and regulation of galectin-3 expression

In human genome galectin-3 is coded by a single gene *LGALS3* which is suited on chromosome 14, locus q21–q22 [104]. Both mouse and human *LGALS3* genes, 10–12 kb and ~17 kb, respectively, are composed of six exons and five introns [105,106]. Exon I encodes the major part of the 5' untranslated sequence of mRNA. Exon II contains the sequence encoding the remaining part of the 5' untranslated region, the translation initiation site and codon sequence for the first six amino acids including the initial methionine. The sequence encoding the N-terminal domain in both, murine and human *LGALS3* was found entirely within exon III. In mouse, the CRD is encoded by three succeeding exons (IV, V, VI), whereas in human, the sequence encoding the CRD was found entirely within exon V. Two transcription initiation sites identified in human *LGALS3* correspond to the β , δ , γ start site region in the mouse gene which also contains the α transcription initiation site. In mouse, the alternative transcription initiation sites lead to two distinct differentially expressed

mRNAs [105,107,108]. Interestingly, the second intron of *LGALS3* contains an internal promoter, which drives production of alternative transcripts [109]. These transcripts arise from an internal gene embedded within *LGALS3*, named galig (galectin-3 internal gene) [110]. They are preferentially expressed in peripheral blood leukocytes, and cannot be used for production of galectin-3 or a modified galectin-3 because they contain two overlapping open-reading frames out-of-frame within the lectin coding sequence. Recently, it was shown that galig is a novel cell death gene encoding mitogaligin, a protein that promotes cytochrome c release upon direct interaction with the mitochondria [111].

The expression of galectin-3, on both transcriptional and translational level is affected by various stimuli. The increase of galectin-3 on both protein and mRNA level was observed in the proliferating fibroblasts comparing to the quiescent cells [84,112]. Furthermore, galectin-3 expression could be considered as a transformation marker since the galectin-3 mRNA content is increased in fully *ras*-transformed fibroblasts, with maximal expression occurring when cells have lost their growth anchorage-dependence [113].

Galectin-3 expression was suggested to be also differentiation marker for certain cell types. Thus, for example, the differentiation of the human promyelocytic cell line HL-60 to macrophage-like cells induced by phorbol ester is accompanied by elevation of both galectin-3 [114] and galectin-3 mRNA level [115]. As well, in vitro differentiation of human monocytes to macrophages provokes significant increase of galectin-3 expression [99]. In contrast, differentiation of dendritic cells from bone marrow progenitors is accompanied by decrease of galectin-3 expression [98].

Galectin-3 is also considered a “macrophage activation marker” due to the fact that its expression is up-regulated in phagocytic macrophages [116]. In addition, the activation of monocytic THP-1 cells provoked by phorbol ester or low density lipoproteins induces galectin-3 expression [117] as well as the exposure of bone marrow-derived macrophages to 1,25-dihydroxyvitamin D3 [118]. In microglia and macrophages exposed to granulocyte-macrophage colony-stimulating factor [100,119], as well as in macrophages and microglia activated by phagocytosis of myelin [120], galectin-3 expression was also elevated. On the other hand, activation of human monocytes by lipopolysaccharide and interferon- γ is accompanied by decrease of galectin-3 expression [99]. The reduced expression of galectin-3, on both protein and mRNA level was also observed in monocytic THP-1 cells treated with non-steroidal [121] or corticosteroidal anti-inflammatory drugs [122] as well as in peritoneal macrophages obtained from animals exposed to acute immobilization stress [123].

Interestingly, in opposite to its wide cell and tissue distribution, galectin-3 is absent or only sparsely expressed in resting lymphocytes and several lymphoid cell lines [66,124,125]. However, the activation of T cells by antibody cross-linking of CD3 or concanavalin A induces galectin-3 expression which is enhanced by cytokines such as interleukins IL-2, -4 and -7 [125]. As well, viral infection of certain T cell lines with human T lymphotropic virus-I (HTLV-I) [126] or

human immunodeficiency virus-1 (HIV-1) [127] induces expression of galectin-3. The activation of B lymphocytes with IL-4 and CD40 cross-linking, signals that promote survival and block final differentiation of the cells, is also accompanied by significant increase of galectin-3 expression [128]. In addition, this study showed that B cells from *Trypanosoma cruzi*-infected mice express galectin-3.

Although a large body of reported data about galectin-3 expression is available in the literature, the mechanisms of regulation of galectin-3 expression are still poorly understood. The promoter region of the human *LGALS3* gene contains several regulatory elements: five putative Sp1 binding sites (GC boxes), five cAMP-dependent response element (CRE) motifs, four AP-1- and one AP-4-like sites, two NF- κ B-like sites, one *sis*-inducible element (SIE) and a consensus basic helix–loop–helix (bHLH) core sequence [106]. The presence of multiple GC box motifs for binding ubiquitous expressed Sp1 transcription factor is a common feature of constitutively expressed, so-called housekeeping genes. The activation of the Sp1 binding transcription factor was suggested to be responsible for galectin-3 induction by Tat protein of HIV [129]. On the contrary, the expression of galectin-3 in serum-starved, quiescent fibroblasts can be induced by addition of serum, on both protein [84] and mRNA level [112], what is a feature of immediately early gene. The SIE that binds *sis*-inducible factors was suggested to be a possible candidate for the growth-induced activation of *LGALS3* expression, caused by the addition of serum.

The presence of CRE and NF- κ B-like site in the promoter region implies that the activation of galectin-3 expression could be also regulated through the signaling pathways involving the cAMP-response element-binding protein (CREB) or the NF- κ B transcription factor. Indeed, the activation of the *LGALS3* expression by the Tax protein during HTLV-I infection of T cells, was shown to independently involve the CREB/ATF and the NF- κ B/Rel transcription factors pathways [126]. We and the others additionally confirmed the involvement of the NF- κ B transcription factor in regulation of galectin-3 expression, as well as the Jun protein, a component of AP-1 transcription factor [117,130]. The regulation of galectin-3 expression through the NF- κ B transcription factor was shown to be mediated by nucling, a novel apoptosis-associated protein, which interferes with NF- κ B via the nuclear translocation process of NF- κ B/p65, thus inhibiting galectin-3 expression on both protein and mRNA level [131].

The galectin-3 promoter was shown to be up-regulated by hepatitis B virus X protein [132] while the regulation of galectin-3 expression in skeletal tissues is mediated by the transcription factor Runx2 [86,133]. Very recent data indicated that in pituitary as well as in other tumors, galectin-3 expression is regulated in part by methylation of CpG islands in promoter region [134]. Taken together, it is evident that the regulation of galectin-3 expression is a complex, fine-tuned mechanism that involves numerous transcription factors and signaling pathways, and which depends on cell type, external stimuli and environmental conditions.

5. Functional properties of intracellular galectin-3

The nuclear and cytoplasmic localization of galectin-3 is well established (reviewed in [135]). However, its localization strongly depends on various factors such as cell type and proliferation status of the cell [84,136–139], cultivation conditions [140] and neoplastic progression [67,141–144] and transformation [56]. On the other hand, biological roles of galectin-3 are defined by its cellular localization.

5.1. Galectin-3 in cytoplasm

The presence of galectin-3 in cytoplasm is well documented for long time [9], yet available information on its biological roles in that compartment were until recently rather scarce. Advanced techniques and technologies of molecular biology, biochemistry, genetics, and bioinformatics enabled studies of yet unrevealed functional properties of galectin-3, thus giving completely new insight on galectin-3 biological roles.

Numerous cytosolic molecules were identified as galectin-3 ligands (Fig. 1). Their diverse biological roles imply the involvement of galectin-3 in various intracellular events. The first cytosolic molecule identified as a galectin-3 ligand *in vivo* was Bcl-2, a molecule involved in regulation of apoptosis [39]. It was suggested that galectin-3 binds Bcl-2 through its carbohydrate-recognition domain, since the interaction is inhibitable by lactose and Bcl-2 binds recombinant C-terminal part of galectin-3 which contains the CRD. Several other molecules involved in apoptotic signaling pathway have been recently identified as novel galectin-3 binding-partners. Using co-immunoprecipitation and confocal microscopic analysis, it was shown that galectin-3 interacts with CD95 (APO-1/Fas), a member of the death receptor family [145]. Nucling, a protein involved in regulation of apoptosis, was identified as a novel galectin-3 binding molecule using the yeast two-hybrid method screen of a mouse embryo cDNA library, immunoprecipitation and immunofluorescence analysis [131]. Alix/AIP1, another cytosolic protein involved in regulation of apoptotic events, was also identified as a galectin-3 binding-partner from a Jurkat cell cDNA library by the yeast two-hybrid method [146].

The involvement of cytosolic galectin-3 in regulation of cell proliferation, differentiation, survival, and death was additionally confirmed by the findings that it affects K-Ras protein [57,147] and Akt protein [148,149]. Galectin-3 was shown to be a selective binding-partner of activated K-Ras (K-Ras-GTP), while the direct interaction of galectin-3 and Akt was not indicated (see below).

In the cytoplasm of human breast epithelial cells galectin-3 interacts with synexin (annexin VII), a 51-kDa Ca^{2+} - and phospholipids-binding protein [150]. Synexin was initially identified as galectin-3-binding protein by using a two-hybrid system, and direct interaction with galectin-3 was confirmed by glutathione S-transferase pull-down assay *in vitro*. Synexin down-regulation provoked by antisense oligonucleotide inhibited translocation of galectin-3 to the perinuclear mitochondrial membrane, thus abolishing its anti-apoptotic activity. These findings revealed mitochondria as a new localization site of

galectin-3 and suggested involvement of synexin in intracellular trafficking of galectin-3.

Some cytokeratins bearing terminal α 1,3 linked N-acetylgalactosamine residue were suggested to be possible galectin-3 ligands in cytoplasm [151]. *In vitro* conditions, it was shown that galectin-3 binds cytokeratins through their glycan parts since these interactions were sensitive to periodate oxidation or α -N-acetylgalactosaminidase treatment of the cytokeratins and inhibitable by glycoconjugates bearing terminal N-acetylgalactosamine. However, up today no data about the interactions of galectin-3 and cytokeratins *in vivo* were collected, so it remains unclear whether cytokeratins are biologically relevant counterparts of galectin-3.

Using a yeast two-hybrid screen of a murine 3T3 cell cDNA library with galectin-3 as the bait, Chrp, a cysteine- and histidine-rich cytoplasmic protein was identified as a galectin-3-binding partner [55]. Yet, confocal immunofluorescence microscopy revealed a distinct difference in the localization of galectin-3 and Chrp; while both proteins were slightly disseminated throughout the cytoplasm, Chrp was strikingly concentrated at the nuclear envelope in a concentric ring. The later study confirmed that galectin-3 directly binds Chrp through its CRD, but also showed that interaction with Chrp does not interfere with galectin-3 binding to polygalactosamine chains of laminin, suggesting a use of two independent binding site within the CRD [152].

5.2. Nuclear galectin-3

The nuclear localization of galectin-3 is well documented, although it lacks any well-characterized nuclear localization signal and the mechanism by which the protein is sequestered in nuclei is unknown. Moreover, there are some inconsistencies regarding the minimal protein sequence required for nuclear localization of galectin-3. Using mutagenesis studies Gong and collaborators showed that deletion of the first 11 amino acids (following the first methionine) results in altered cellular compartmentalization and loss of nuclear localization [35]. Furthermore, when that sequence was fused to green fluorescent protein (GFP), the fluorescence of the reporter was predominantly localized in the nucleus. In contrast to these findings, later study rather implied involvement of the CRD in nuclear import and retention of galectin-3 inside the nuclei [22]. Using confocal immunofluorescence microscopy and immunoblot analysis the authors showed that in COS-7 and Rb-1 cells transfected with cDNA encoding mutants of hamster galectin-3 lacking the first 103 amino acids (Δ 1–103), truncated galectin-3 localizes in nuclei, while further deletion (additional 7 amino acids, Δ 1–110) completely abolishes its nuclear localization. However, amino acid specificity of sequence A¹⁰⁴PTGALT¹¹⁰ is not essential for nuclear localization, since mutants Δ 23–110, Δ 35–110 and Δ 104–110, lacking this sequence were also found in the nuclei of the cells. Taken together, it remains unclear what is a minimal amino acid sequence required for nuclear localization. Still, it is interesting to observe that some cell types in spite of the intense cytoplasmic localization, do not express galectin-3 in the nucleus [153,154], even when it is

overexpressed [22], implying the importance of specific cell physiology for particular cellular localization as well.

In opposite to the unsolved mechanism of galectin-3 import into the nucleus, the export from the nucleus is rather elucidated. Galectin-3 export is rapid and selective process that proceeds via leptomycin-inhibitable pathway [155]. Leptomycine is a drug shown to disrupt the interaction between leucine-rich nuclear export signal (NSE) (residues 241–249 of the murine galectin-3 sequence), highly conserved in the galectin-3 homologues of various species, and chromosome maintenance region 1 (CRM1), a nuclear export receptor.

In nucleus galectin-3 was shown to be associated with ribonucleoprotein complexes [53]. It acts as a pre-mRNA splicing factor and is involved in spliceosome assembly [156]. In contrast to the previous reports which showed binding of galectin-3 to single-stranded DNA (ssDNA) and RNA in a lactose-independent manner [54], more recent data suggested indirect interaction between galectin-3 and pre-mRNA, presumably through protein complexes containing Gemin4, which was shown to be intranuclear counterpart of galectin-3 [157].

Lin et al. [158] recently implicated the role of nuclear galectin-3 in regulation of gene transcription. Through enhancement or stabilization of transcription factor binding to the CRE and possibly Sp1 sites in the cyclin D₁ promoter region, galectin-3 was shown to promote trans-activation functions of transcription factors CREB and Sp1 and to induce cyclin D₁ promoter activity. Since CRE and Sp1 elements are present in promoter regions of many genes, it is reasonable to speculate that these proteins may not be the only ones which expression is regulated by galectin-3. Possible candidates are cyclin A, cyclin E, p21^{WAF1/CIP1}, and p27^{KIP1}, since previously report documented that overexpression of galectin-3 modulates their expression in human breast epithelial cells [159]. The role of galectin-3 in regulation of gene transcription was additionally confirmed by the findings that in papillary thyroid cancer cells galectin-3 directly interacts with the homeodomain of the thyroid-specific transcription factor 1 (TTF-1) and causes stimulation of the DNA-binding activity of TTF-1. In that way up-regulates the transcriptional activity of TTF-1 thus contributing to the proliferation of the thyroid cells [56].

In addition, β -catenin, a molecule involved in Wnt signaling pathway, was also identified as a novel binding partner of galectin-3 in nucleus [58]. The most recent study showed that axin, a regulator protein of Wnt pathway, that complexes with β -catenin and enhances its GSK-3 β -dependant phosphorylation, also binds galectin-3 using the same sequence motif (S⁹²XXXS⁹⁶), and promotes GSK-3 β -dependent phosphorylation of galectin-3 [59]. These findings additionally support previous suggestion that galectin-3 plays an important role in regulation of Wnt/ β -catenin signaling pathway.

Nuclear galectin-3 also interacts with a glucose-binding lectin (CBP70), 70 kDa glycoprotein [160], which is the only counterpart of galectin-3 in nucleus, identified thus far, that interacts with galectin-3 in a lactose-dependent manner.

The biological functions of galectin-3 in nucleus are reviewed in details by Patterson et al. [161].

6. Functional properties of extracellular galectin-3

Galectin-3, as all other vertebrate galectins, lacks recognizable secretion signal sequence and does not pass through the standard ER/Golgi pathway [6]. Still, numerous findings confirmed its extracellular localization; galectin-3 was found on the cell surfaces and in the extracellular matrix, bound to its numerous extracellular counterparts, in biological fluids and sera, as well as in culture media of certain cell lines. Furthermore, extracellular galectin-3 was shown to mediate cell adhesion and signaling. R. C. Hughes and his collaborators as well as some other groups provided strong evidences imposing an alternative secretory pathway for galectin-3 export from the cell, which was suggested to be mediated by specific vesicles, although such bodies have not yet been identified [6,34,72,153,154,162,163]. Consequently, in the proposed secretory mechanism, space segregation prevents interaction of galectin-3 and its potential glycosylated counterparts secreted by ER/Golgi apparatus, before reaching their final destination outside of the cell. Another secretory pathway of galectin-3 has been recently implicated; galectin-3 was shown to be a component of exosomes secreted by dendritic cells [164,165]. Exosomes are small (60–90 nm) antigen-presenting and immunostimulating vesicular bodies formed by inward budding of endosomal membrane into the lumen of the endosomes and exported into the extracellular space. In both proposed cases, the vesicles containing galectin-3 can be exported outside of cell, and after their lysis, released galectin-3 can interact with components of extracellular matrix and/or its membrane counterparts. Alternatively, vesicles might directly fuse with other cells, thus resulting in galectin-3 uptake by these cells.

Galectin-3 can be rapidly internalized from the extracellular compartment by endocytosis in lactose-dependent manner, as observed in tumor cells [166]. Furthermore, it mediates endocytosis of β 1 integrin (CD29) via a caveolae-like pathway [166] as well as endocytosis of AGE products and acetylated-low density lipoproteins [167].

The extracellular galectin-3 exhibits numerous autocrine and paracrine effects (Fig. 2). It mediates cell adhesion and cell activation and acts as a chemoattractant for certain cell types. In that way, galectin-3 affects various biological processes such as maintenance of cellular homeostasis, immune reactions, organogenesis and angiogenesis, and tumor invasion and metastasis (reviewed in [102,168–172]). However, it should be mentioned that many studies were performed using exogenously introduced galectin-3 in high concentrations, thus biological functions of galectin-3 in physiological conditions remain to be elucidated.

6.1. Modulation of cell adhesion

The effects of galectin-3 as a modulator of cell adhesion are based on its multivalent properties and the ability to bind cell surface glycoproteins and glycosylated components of extracellular matrix. Galectin-3 was shown to bind laminin [31,173,174] and fibronectin [44], as well as hensin [175], elastin [176], collagen IV [177] and tenascin-C and -R [178].

In addition, galectin-3 binds certain integrins, the main membrane molecules involved in cell adhesion, in sugar-dependent manner. $\alpha 1\beta 1$ integrin [177] and α subunit (CD11b) of $\alpha M\beta 1$ integrin (CD11b/18, Mac-1 antigen), present on the surface of the macrophages, [179] were shown to be galectin-3 receptors. Galectin-3 also binds the heavy chain of CD98, a 125-kDa heterodimeric membrane glycoprotein present on human and mouse monocytes/macrophages and on activated T cells thus mediating CD98 dimerization, which in turn can promote integrin activation, as proposed by Hughes [180].

Galectin-3 can inhibit or potentiate cell adhesion of different cell types on extracellular matrix proteins; it promotes adhesion of human neutrophils to laminin [174] and to endothelial cells [181], and mediates binding of L-selectin-triggered lymphocytes to dendritic cells [182]. In addition, the overexpression of galectin-3 significantly enhances the adherence of the human breast cancer cell line Evsa-T to laminin, fibronectin and vitronectin [183]. On the other hand, galectin-3 can act as a de-adhesion molecule, as observed in thymus, where endogenously produced galectin-3 disrupts thymocyte/microenvironmental cell interactions [74]. The interactions of galectin-3 with some other glycoproteins, such as the lysosomal membrane glycoproteins Lamp-1 and -2 [179], carcinoembryonic antigen [184], colon cancer mucin [185] and C4.4A, a GPI-anchored glycoprotein [186] were suggested to be involved in adhesion of cancer cells to extracellular matrix. In addition, the evidence that surface galectin-3 mediates homotypic cell adhesion by bridging through branched, soluble complementary glycoconjugates, implies its role in aggregation of tumor cells in the circulation during metastasis [187].

6.2. Cell activation and chemoattraction

It is well known that cross-linking of surface proteins can trigger signal transduction cascades which in turn induce numerous biochemical reactions in the cells, thus resulting in their activation. A cross-linking of glycosylated membrane receptors is often mediated via binding of their glycan parts and multivalent lectins, such as galectin-3. Indeed, there are many evidences of the involvement of galectin-3 in activation of various cell types, particularly of those participating in immune reactions (Fig. 3).

Galectin-3 potentiates lipopolysaccharide (LPS)-induced production of interleukin-1 (IL-1) [188] and triggers the production of superoxide anion by human peripheral blood monocytes [99] and neutrophils [189]. It also activates NADPH-oxidase in human neutrophils primed by in vivo exudation into skin blister lesions, but not in peripheral blood neutrophils [190]. Nevertheless, galectin-3 can induce a respiratory burst in human peripheral blood neutrophils, after the cell exposure to the stimuli, such as formyl-methionyl-leucyl-phenylalanine (fMLP) [190] or LPS [191] that mobilize intracellular organelles, and cause expression of the glycoproteins related to the organelles on the cell surface. The increase in oxidative response is paralleled by an increased binding of galectin-3 to the surface of the cells, possibly through CD66a

and CD66b that were suggested to be functional receptors of galectin-3 in neutrophils [192]. It was recently shown that galectin-3 increases phagocytic activity and CD66 surface expression on human neutrophils [193]. Moreover, pre-incubation with galectin-3 primes neutrophils to the effects of soluble fibrinogen, thus resulting in a synergistic action on degranulation.

As well, galectin-3 induces mediator release by mast cells [95], possibly through the cross-linking of Fc ϵ RI and/or Fc ϵ RI–IgE complex, since both Fc ϵ RI and IgE were shown to be galectin-3 binding-partners. The exposure of human Jurkat T cells to galectin-3 activates the cells, as evidenced by IL-2 production [126], and it also acts as a mediator of IgE production in B lymphocytes provoked by polymorphonuclear leukocytes in IgE-associated atopic eczema/dermatitis syndrome [194].

Besides the positive effects, the suppressive action of galectin-3 on myeloid cells was also observed. The exposure of human eosinophils, peripheral blood mononuclear cells (PBMC), an eosinophilic cell line, and an antigen-specific T cell line to galectin-3 results in a selective inhibition of IL-5 expression, on both protein and mRNA level [195]. Since down-regulation of the IL-5 gene expression was not observed in PBMC from Fc γ RII-deficient mice, Fc γ RII was suggested to be a galectin-3 receptor which is engaged in signal transduction pathway resulting in inhibition of IL-5 gene transcription [196].

Some other cell types were also shown to be affected by galectin-3. For example, galectin-3 was shown to induce endothelial cells morphogenesis and angiogenesis in vitro and in vivo [197]. In addition, the induction of endothelial cell motility and multicellular network formation in vitro and the stimulation of corneal angiogenesis in vivo provoked by NG2, a transmembrane chondroitin sulfate proteoglycan, was shown to be mediated by formation of NG2–galectin-3– $\alpha 3\beta 1$ integrin complex, suggesting a mechanism by which galectin-3 may induce angiogenesis [198]. As well, galectin-3 was shown to activate cardiac fibroblasts [199] and to be increased in synovial fibroblasts from rheumatoid arthritis patients after adhesion to cartilage oligomeric matrix protein [200]. It was also suggested that endogenously galectin-3 generated by activated hepatic stellate cells, in autocrine manner, can induce their proliferation via protein kinases C- and A-dependent signaling pathway [101]. Since galectin-3 was shown to cross-link Mgat5-modified N-glycans on EGF and TGF- β , thus delaying their removal by constitutive endocytosis, it is reasonable to speculate that besides Mgat5 activity, the presence of extracellular galectin-3 can be determining factor for prolonged cell activation [52]. On the other hand, the extracellular galectin-3 can act as a suppressor of cell activation as suggested by findings that galectin-3 cross-linking of Mgat5-modified N-glycans on T cell receptors (TCR) prevents uncontrolled activation of T cells [51].

In addition to other roles, the extracellular galectin-3 was found to act as a chemoattractant for monocytes and macrophages, as well as alveolar macrophages [201]. In vivo and in vitro studies showed that galectin-3 induces migration of the cells and that both, the N-terminal domain and the CRD are

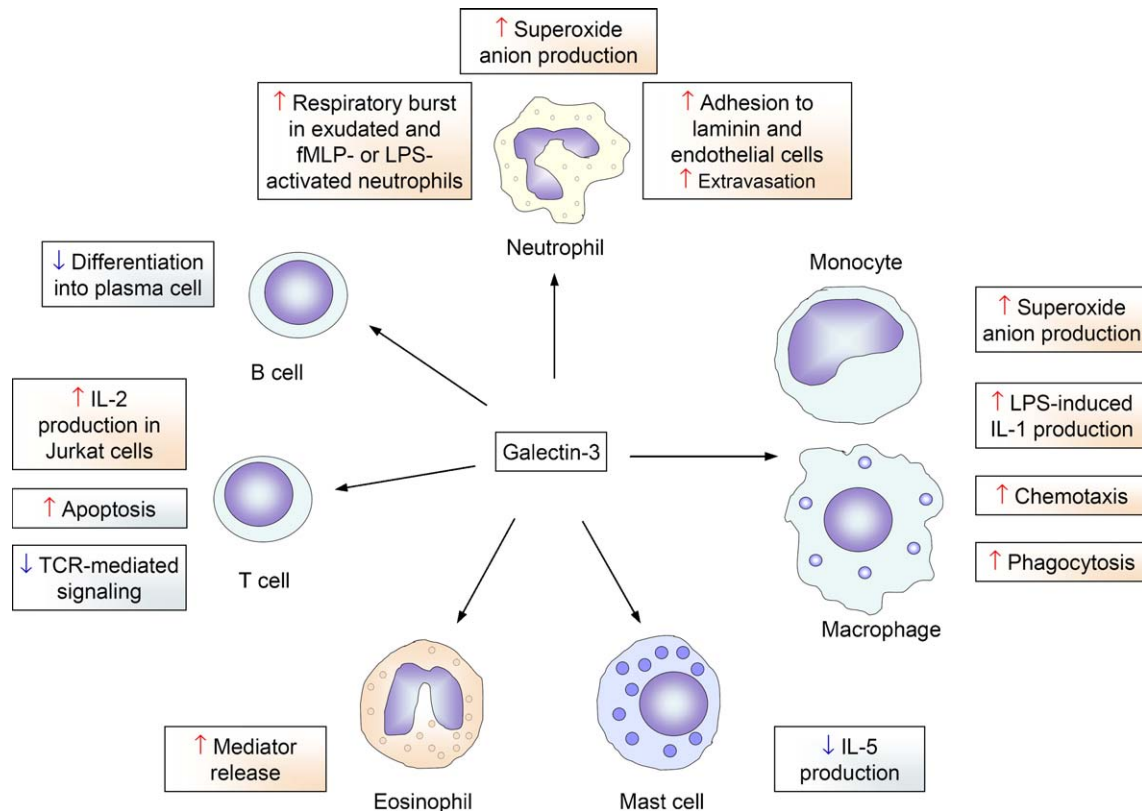


Fig. 3. The effects of galectin-3 on immune cells. Red upwards arrows indicate positive effects, blue downwards arrows indicate negative effects. Pro-inflammatory effects of galectin-3 are indicated in red shaded boxes, anti-inflammatory effects are indicated in blue shaded boxes. fMLP—formyl-methionyl-leucyl-phenylalanine IL-1, -2, -5—interleukin-1, -2, -5, LPS—lipopolysaccharide, TCR—T cell receptor.

required for that activity. While higher concentrations of galectin-3 are necessary for chemotactic effects (1 μM), in lower concentrations (10–100 nM) galectin-3 provokes chemokinesis, i.e., increased non-directional cell movements. Chemotactic, but not chemokinetic activity of galectin-3 was suggested to be mediated by the G-protein-coupled cell surface receptors. In addition, galectin-3 induces Ca^{2+} influx in monocytes that is inhibitable by lactose.

7. Galectin-3—a role in life and death interplay

There are numerous data on galectin-3 roles in regulation of cellular homeostasis. This protein was shown to modulate cell growth, to control cell cycle and to be involved in regulation of apoptosis. Some of the data were obtained using exogenously added galectin-3, simulating the effects of the extracellular galectin-3 while those, regarding the effects of intracellular galectin-3, were demonstrated by ectopically expressing the protein, inhibiting *LGALS3* expression, or using specific inhibitors. In addition, very valuable information were obtained using galectin-3 double mutant mice (*gal^{-/-}*).

7.1. Cell growth and differentiation

Proliferation and differentiation of certain cell types, as it was mentioned above, is accompanied by increased or suppressed expression of galectin-3. In addition, both endog-

enous and exogenous galectin-3 can induce or inhibit cell growth, or direct cell differentiation in a specific way.

Galectin-3 was shown to induce outgrowth of neurites from dorsal root ganglia explants [202] and to stimulate mesangial cell proliferation [80]. As well, when galectin-3 is added to quiescent cultures of normal human lung fibroblasts it stimulates DNA synthesis and induces cell proliferation [203]. Since these effects are inhibitable by lactose, the property of galectin-3 to act as a mitogen is ascribed to its lectin activity. In addition to the aforementioned effects of extracellular galectin-3 on the cell activation through affecting life-time of cytokine receptors, it is reasonable to believe that galectin-3 in the same way can modulate cell growth.

Galectin-3 can also act as a negative growth regulator. The exogenously added galectin-3 inhibits granulocyte-macrophage colony-stimulating factor (GM-CSF)-induced proliferation of rat bone marrow cells [204]. The findings that galectin-3 suppresses cell growth of MDCK but not of mutant MDCK cells, defective in galactose transport, which lack extracellular receptors for galectin-3, suggest that the inhibitory effects of galectin-3 are mediated through its interactions with appropriately glycosylated surface receptors [205].

A role of endogenous galectin-3 in regulation of cell growth mainly depends on the type of the cell. In certain cell types, galectin-3 promotes cell growth and proliferation, while in some of them it has the opposite effects. The human T lymphoma cell line Jurkat transfected with galectin-3 display higher growth

rates than control transfectants [39]. Furthermore, the suppression of galectin-3 expression by antisense oligonucleotides inhibits proliferation of mitogen-activated T cell [125]. As well, blocking of its expression in highly malignant human breast carcinoma MDA-MB-435 leads to significant suppression of tumor growth [206], as well as anchorage-independent growth of the human papillary thyroid carcinoma cell line [207].

On the contrary, galectin-3 can be a suppressor of cell growth as observed in the study of Ellerhorst et al. [208]. The authors demonstrated that stably transfected galectin-3-expressing cell lines, developed from the prostate cancer cell line LNCaP, which does not constitutively express this molecule, proliferates at a slower rate *in vitro* than either the vector control-transfected lines or parental LNCaP cell line.

These findings impose the question about the mechanism by which endogenous galectin-3 affects cell growth and proliferation. According to available data, it seems that galectin-3 is able to modulate specific signal transduction pathways through the interaction with particular signaling molecules. Thus, as it was previously mentioned, galectin-3 preferentially binds activated K-Ras, the most important Ras oncoprotein in human tumors [57]. This interaction determines the strength, duration, and selectivity of the K-Ras signal; galectin-3 seems to increase K-Ras signaling to phosphatidylinositol-3 kinase (PI3-K) and to Raf-1 as well as to a third, still unknown effector pathway that attenuates extracellular signal-regulated kinase (ERK) activation. These findings suggest that level of galectin-3 defines outputs of oncogenic K-Ras protein. Furthermore, very recent data confirmed that galectin-3 stimulation of cell proliferation, and anchorage-independent growth, as well as anti-apoptotic activity involves K-Ras/MEK (mitogen-activated protein/ERK kinase) pathway [147]. In addition, through the interaction with the thyroid-specific transcription factor TTF-1, galectin-3 was shown to contribute proliferation of papillary thyroid cancer cells [56]. Galectin-3 also interacts with the T cell factor (Tcf)-dependent transcription complex; it binds to β -catenin/Tcf complex, co-localizes with β -catenin in the nucleus, induces the transcriptional activity of Tcf-4, and affects β -catenin stimulation of cyclin D₁ and *c-myc* expression, thus promoting cell growth and proliferation [58]. Finally, recent findings showing that galectin-3 can affect activation of Akt, a kinase that regulates cell proliferation and apoptosis in cancer cells, in both ways, by suppressing [148] and by stimulating [149] Akt activity, but in two different cell types, suggest that the effects of galectin-3 on the cell growth and proliferation strongly depends on the properties of the particular cell type.

Galectin-3 was also shown to be a modulator of cell differentiation. The activation of B lymphocytes by IL-4 that direct differentiation into memory cells is accompanied by increase of galectin-3 expression [128]. A down-regulation of the transcription factor Blimp-1 has been proposed to be a mechanism underlying IL-4-induced blockade of plasma cell differentiation. When galectin-3 expression in B cells was suppressed by using the antisense strategy, IL-4 stimulation failed to down-regulate Blimp-1 transcription factor, resulting in differentiation toward a plasma cell pathway. According to these data it seems that galectin-3 participates in IL-4-induced

down-regulation of Blimp-1 transcription factor, thus contributing in the differentiation of B cell into memory cells. As well, galectin-3 was recently suggested to be a negative regulator of terminal differentiation of hypertrophic chondrocytes, possibly by acting as an anti-apoptotic extracellular matrix protein that maintains extracellular matrix anchorage [209].

7.2. Apoptosis

Both pro- and anti-apoptotic activities of galectin-3 were observed. In general, intracellular galectin-3 acts as an anti-apoptotic factor, whereas the extracellular galectin-3 mainly acts as a pro-apoptotic factor. The anti-apoptotic activity of the extracellular galectin-3 can also be assumed, though direct evidence is still lacking.

It is well established that the intracellular galectin-3 protects different cell types against apoptosis in response to various stimuli. Thus, for example galectin-3-transfected Jurkat cells are more resistant to apoptosis provoked by anti-Fas antibody and staurosporine compared to the controls [39]. As well, when galectin-3-negative Burkitt lymphoma cells were transfected with a galectin-3 expressing plasmid, resistance to anti-Fas-induced cell death was also markedly increased [210]. The ectopic expression of galectin-3 in human breast carcinoma BT549 cells that innately expresses no galectin-3, makes them more resistant to apoptosis induced by cis-diamminedichloroplatinum (cisplatin) [211], and anoikis (apoptosis caused by loss of cell anchorage) [159] comparing to the controls. Galectin-3 overexpression in another human breast carcinoma cell line Evsa-T protects cells from apoptosis induced by cycloheximide/TNF- α and UVB irradiation [183]. The role of galectin-3 in protection of macrophages against apoptosis was demonstrated in the study of the effects of apoptotic stimuli on peritoneal macrophages from galectin-3-deficient mice in which was shown that these cells undergo apoptosis more rapidly comparing to the cells obtained from wild-type mice [212]. Quite the opposite, Lee et al. [148] demonstrated pro-apoptotic activity of galectin-3, showing that the overexpression of galectin-3 potentiates tumor necrosis factor (TNF)-related apoptosis-inducing ligand, TRAIL-induced cytotoxicity.

Although the anti-apoptotic effect of the intracellular galectin-3 is well documented, the precise mechanism of this action is still elusive. Yet, it seems that several regulatory pathways are involved. In the response to a variety of apoptotic stimuli, galectin-3 translocates from the cytosol or the nucleus to the perinuclear mitochondrial membrane, prevents mitochondrial damage and inhibits cytochrome c release, thus down-regulating caspase activation [150]. Since the translocation of galectin-3 to the mitochondria is mediated by synexin, availability and functionality of synexin could be a limiting factor in galectin-3 anti-apoptotic activity.

The phosphorylation of galectin-3 at position Ser⁶ seems to be essential for its anti-apoptotic activity since the transfection of galectin-3-null human breast carcinoma BT549 cells with the mutant galectin-3 (S6A and S6E) fails to protect the cells from cisplatin-induced cell death comparing to the transfection with the wild type galectin-3 [36,213]. Additionally, it was suggested

that anti-apoptotic effect of galectin-3 is at least in part mediated by the activation of ERK and JNK (c-Jun NH₂-terminal kinase) pathways [213].

It was aforementioned that galectin-3 binds Bcl-2 [39]. Although galectin-3 is not a member of the Bcl-2 gene family, these molecules share significant sequence similarity; there is 28% identity and 48% similarity between protein sequences of galectin-3 and Bcl-2. The N-terminal domains of both molecules are rich in proline, glycine, and alanine, whereas the CRD of galectin-3 contains the NWGR motif (residues 180–183) which is highly conserved within the BH1 domain of Bcl-2 family members (residues 143–146 in human Bcl-2). Galectin-3 is the only member of the galectin family, known by now, that contains the NWGR motif and that acts as anti-apoptotic molecule. In fact, the NWGR motif was shown to be essential for anti-apoptotic activity of galectin-3, since the mutagenesis study showed that substitution of glycine with alanine (G182A) abrogates that galectin-3 function [211].

The anti-apoptotic effects of galectin-3 in T cell are possibly mediated through its interaction with CD95 (APO-1/Fas) receptor, a member of the death receptor family [145]. The activation of CD95 triggers a sequential activation of several caspases, thus mediating apoptosis-signaling pathway initiated by engagement of Fas. Depending on the signaling response initiated by activation of CD95, two types of CD95-mediated apoptotic pathway can be distinguished. It was shown that in type I cells the formation of the death-inducing signaling complex (DISC) and the production of activated caspase-8 is very efficient, while in type II cells is poor. Since transfection of galectin-3 cDNA into galectin-3 null cells (type II) results in converting them to type I apoptotic phenotype, it was suggested that galectin-3 modulates CD95-mediated apoptotic signaling pathway and determines which one of the two phenotypes (I or II) the cell will develop.

Tumor necrosis factor (TNF)-related apoptosis-inducing ligand, TRAIL, a pro-apoptotic cytokine, belongs to a family of ligands that transduce death signals through death domain-containing receptors. Akt, a serine/threonine kinase was assumed to be involved in resistance to TRAIL-mediated apoptosis observed in certain types of tumors. As mentioned before, the overexpression of galectin-3 in the human breast carcinoma cell line BT594 promotes TRAIL-induced cytotoxicity in a way to suppress Akt activity by dephosphorylation [148]. On the contrary, Oka and collaborators [149] showed that in the human bladder carcinoma cells J8 which overexpress galectin-3, the level of constitutively active Akt is high and that the cells are resistant to TRAIL-induced apoptosis. The authors also proposed the model of the role of galectin-3 effect on Akt during apoptosis; galectin-3 promotes activation of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway, which blocks loss of the mitochondrial membrane potential, resulting in inhibition of caspase-9 and caspase-3 activation and suppression of apoptosis. It is reasonable to assume that observed inconsistencies are probably due to the use of different cell lines expressing different galectin-3-associated proteins.

Several apoptotic signals induce expression of nucling, a pro-apoptotic molecule which was shown to interact with

galectin-3 in cytoplasm, and to down-regulate the expression of galectin-3 on both mRNA and protein level [131]. In addition, nucling-deficient cells, in which galectin-3 expression is up-regulated, appeared to be resistant to some forms of pro-apoptotic stress. These findings suggest that nucling can modulate galectin-3 anti-apoptotic activity on two levels, by direct interacting with galectin-3 and by suppressing galectin-3 synthesis.

Although until now there is no direct evidence of anti-apoptotic activity of the exogenously added galectin-3, it is reasonable to believe that the extracellular galectin-3 might contribute to the survival of the cells exposed to apoptotic stimuli since it acts as a mitogen and modulates cell adhesion to extracellular matrix that generally promotes protection of the cells from apoptosis. The findings that the overexpression of galectin-3 in human breast carcinoma cells significantly enhances adhesion to the components of the extracellular matrix, thus promoting survival of the cell exposed to different apoptotic stimuli [183], contribute aforementioned assumption.

On the other hand, pro-apoptotic activity of the extracellular galectin-3 was observed in several cell types, such as human T leukemia cell lines, human peripheral blood mononuclear cells, and an activated mouse T cells [214]. Interestingly, the T cell lines expressing galectin-3 (SKW6.4 and H9) were less sensitive to apoptosis induced by exogenous galectin-3 comparing to the galectin-3-null T cell lines (Jurkat, CEM and MOLT-4). These differences might be a consequence of the anti-apoptotic activity of intracellular galectin-3 in galectin-3 expressing cells that opposes to the apoptotic action of the extracellular galectin-3. The cell surface glycoproteins CD29 and CD7 were identified as galectin-3 receptors related to activation of intracellular apoptotic signaling. Galectin-3 binding to CD29/CD7 complex triggers signaling cascades that provokes activation of mitochondrial apoptosis events including cytochrome c release and caspase-3 activation. Therefore, galectin-3 secreted by tumor cells can contribute to immune escape mechanism during tumor progression through induction of apoptosis of cancer-infiltrating T-cells [215].

7.3. Cell cycle

The regulation of cell cycle is complex and puzzling process that involves numerous proteins, including galectin-3. The expression of galectin-3 was shown to be dependent on the cell cycle, but galectin-3 can also affect the progression of the cell cycle.

The human breast epithelial BT549 cells innately undergo apoptosis induced by the loss of cell anchorage (anoikis), but galectin-3-overexpressing BT549 cells respond to the anoikis by G1 arrest without detectable cell death [159]. The galectin-3-mediated G1 arrest is accompanied by down-regulation of cyclin E and cyclin A and up-regulation of their inhibitory proteins p21^{WAF1/CIP1} and p27^{KIP1}, as well as cyclin D. Furthermore, retinoblastoma (Rb) protein, which is hyperphosphorylated during S, G2, and most of the M phases, was found to be hypophosphorylated. Similarly, the later study reported by Lin et al. [216] showed that genistein, a cell cycle regulator

effectively induces apoptosis without detectable cell cycle arrest in BT549 cells, while in galectin-3 transfected BT549 cells, this soybean isoflavonoid induces cell cycle arrest at the G2/M phase without apoptosis induction. However, in contrast to the previous study, genistein enhanced p21^{WAF1/CIP1} expression, but abolished galectin-3 induction of p27^{KIP1}, thus leading to G2/M arrest. Taken together, these data suggest that galectin-3 causes cell cycle arrest at different points depending on the apoptotic stimuli, but the precise mechanisms of its action should be clarified.

8. Galectin-3 in immune response

The information on functional properties of galectin-3 summarized in the previous sections strongly suggests the importance of galectin-3 in the regulation of the immune response and inflammation. In general, galectin-3 is a powerful pro-inflammatory signal. Certain cells produce and secrete a large amount of galectin-3 as a response to various inflammatory stimuli. When secreted or externalized, galectin-3 may affect inflammatory cells by an autocrine or paracrine mechanism; it triggers/promotes respiratory burst in neutrophils and monocytes and induces mediator release by mast cells, it promotes adhesion of human neutrophils to laminin and endothelial cells, and acts as a chemoattractant for monocytes and macrophages. Studies in vivo have also provided strong evidences of pro-inflammatory effects of galectin-3 [212,217]. Thus, targeted disruption of galectin-3 gene in mice results in a reduced inflammatory response after induction of peritonitis by injection of thioglycollate, as evidenced by significantly reduced number of granulocytes [217] and less macrophage infiltrations [212] compared to wild-type animals. Galectin-3-deficient (gal^{-/-}) mice also show attenuated phagocytic clearance of apoptotic thymocytes by peritoneal macrophages compared to wild-type mice [218]. In addition, compared to wild-type macrophages, gal^{-/-} cells exhibit reduced phagocytosis of IgG-opsonized erythrocytes and apoptotic thymocytes in vitro. These findings imply the important role of galectin-3 in phagocytosis, particularly in the inflammatory response, although the mechanism of the process is unknown.

Additionally, we may speculate that intracellular galectin-3 could contribute to the persistence of the inflammation by acting as an anti-apoptotic factor in promoting the survival of inflammatory cells.

The importance of galectin-3 in inflammatory response also emerges from the property of galectin-3 to recognize galactose-containing glycoconjugates on pathogens [219]. It was suggested that galectin-3 can bind LPSs of *Klebsiella pneumoniae*, *Salmonella minnesota*, *Salmonella typhimurium*, and *Escherichia coli* [220]. Interestingly, galectin-3 can interact with LPSs via two distinct sites; through its CRD, interacting with β -galactoside containing side-chains in lactose-dependent manner, and through its ND, interacting with a non-glycan structure, lipid A/inner core region of LPS, independently of its lectin activity. Galectin-3 was also implicated in the binding of *Pseudomonas aeruginosa* to corneal epithelial cells [68]. In regard to the ability of galectin-3 molecules to multimerize upon

binding to the specific glycoconjugates, the recognition of LPS by galectin-3 might promote adhesion of pathogens to host cells or extracellular matrix.

Galectin-3 was also shown to interact with glycolipid component of *Mycobacterium tuberculosis* and to accumulate in pathogen-containing phagosomes in macrophages during the course of the infection [221]. In addition, galectin-3-deficient mice exhibit reduced capacity to clear late *Mycobacterium tuberculosis* infection compared to wild-type mice. Galectin-3 was also found to bind *Schistosoma mansoni* eggs possibly through GalNAc β 1,4GlcNAc bearing glycans [222].

Galectin-3 recognizes *Leishmania major*-specific polygalactosyl repeats (Gal β 1,3)_n expressed on the major surface glycoconjugates, lipophosphoglycans [223]. The association of galectin-3 with *L. major* parasites results in the cleavage of galectin-3 and the formation of the truncated molecule which lacks N-terminal domain. The authors implied the possibility that truncated molecules (lacking oligomerization properties) compete with intact galectin-3 molecules for binding to Mgat5-modified N-glycans of leukocyte surface receptors and prevent multi-lattice formation, thus inducing enhanced immune reactions. The aspects of galectin-3 in innate immunity are reviewed in details by Sato and Nieminen [224].

The pro-inflammatory role of galectin-3 was also demonstrated in a murine asthma model [225]. Galectin-3-deficient mice sensitized with ovalbumin develop fewer eosinophils and significantly less airway hyperresponsiveness compared to similarly treated wild-type mice. However, it was previously demonstrated that gene therapy with galectin-3 suppresses airway response in rat asthma model [226]. The intratracheal instillation of galectin-3-expression plasmid inhibits bronchial obstruction and inflammation in antigen-challenged rats through IL-5 gene down-regulation. Taken together, these studies reveal opposite effects of the endogenous galectin-3 and galectin-3 applied in therapeutic manner; while the endogenous protein promotes inflammatory response in asthma, pharmacological application of galectin-3 might suppress it, thus opening a new approach for future treatment of the disease.

As it was described above, galectin-3 was suggested to be possible modulator of T cell activation; it cross-links Mgat5-modified N-glycans on T cell receptor, thus affecting recruitment of TCR complex to the site of antigen presentation. Interestingly, mice deficient in Mgat5 show enhanced delay-type hypersensitivity and increased susceptibility to autoimmune disorders [51]. In addition, anti-galectin-3 autoantibodies were identified in some autoimmune and inflammatory disorders, Crohn's disease [227] and systemic lupus erythematosus and polymyositis/dermatomyositis [228]. Thus, it is possible that galectin-3 plays a role in pathogenesis of autoimmune disease, although the galectin-3-deficient mice have not been reported to experience autoimmunity.

Galectin-3 level was found to be elevated in sera and synovial fluid from rheumatoid arthritis (RA) patients, and it correlated with levels of C-reactive protein [229]. When RA synovial fibroblasts (SF) were cultured in vitro they showed an increased release of galectin-3 into the medium, although intensified protein synthesis was not observed. Recent study

showed that these cells exhibit higher adhesiveness to cartilage oligomeric matrix protein compared to osteoarthritis SF, as well as increased intracellular level of galectin-3 [200]. Consequently, increased expression and secretion of galectin-3 into synovial fluid might additionally promote inflammatory response, by both autocrine and paracrine mechanisms.

In summary, galectin-3 can be positive and negative regulator of inflammatory response, depending on multiple factors, such as specific inflammatory conditions, the type of targeted cell or its expression level.

9. Galectin-3 in cancer biology

There is a large number of published data regarding galectin-3 expression in cancer. Since the obtained results are rather inconsistent, it is not possible to launch any general conclusion about the role of galectin-3 in cancer. These inconsistencies are consequence of (i) different experimental approaches and techniques that had been used in these studies, and (ii) high specificity of each type of cancer. However, all cancers share some common characteristics, such as uncontrolled proliferation, disturbed adhesiveness, and resistance to apoptosis. In regard to functional properties of galectin-3, described above, it is more than clear that this lectin plays multiple roles in cancer pathogenesis, proliferation and spreading of metastasis.

In addition, not only the intensity of galectin-3 expression, but also its intracellular distribution was found to be altered in certain types of cancers. Interestingly, nuclear localization of galectin-3 is mainly related to its anti-tumor effects, whereas its cellular localization correlates with neoplastic progression [67,141–144,230]. However, malignant transformation of thyroid cells is accompanied with intense nuclear localization of galectin-3 [56].

Galectin-3 expression recently emerged as a potential diagnostic and/or prognostic marker of some cancers (reviewed in [103]). Particularly encouraging results were obtained for thyroid cancers, suggesting the usefulness of the measurement of galectin-3 expression in diagnosis of this malignancy [231]. However, some other studies impose caution in interpretation of the obtained results. Although galectin-3 is not a universal and unambiguous marker of thyroid cancers, it could be a helpful parameter in diagnosis of these tumors.

For detailed information on galectin-3 in cancer, the reader is referred to several recent reviews [102,171,172,215,232].

10. Concluding remarks

From available data galectin-3 emerges as a structurally unique and functionally amazing galectin, expressed in various tissues and cell types, and detectable inside and outside of cells, as well as on the cell surfaces. The biological roles of galectin-3 have been initially attributed to its carbohydrate-binding activity, but during the past decade a whole new spectrum of its functions, unrelated to lectin activity has been revealed. Galectin-3 was found to be involved in many biological processes, such as cell–cell and cell–extracellular matrix adhesion, cell growth and differentiation, cell-cycle, signaling,

and apoptosis, as well as in angiogenesis. Consequently, galectin-3 is involved in regulation of development, immune reactions, tumorigenesis, and tumor growth and metastasis.

Valuable information on galectin-3 functions have risen from the studies of galectin-3-deficient mice. Although viable and fertile, these animals exert multiple disorders related to development [233], inflammation response [212,217,218] or function of some organs [234]. Numerous questions call for elucidation. Detailed investigations of various cell populations, tissues and organ systems of galectin-3-null mice are needed to tie up our knowledge of physiological role of galectin-3. In order to clarify the precise mechanisms of galectin-3 actions, it is of utmost importance that its physiologically functional ligands are identified. The synthesis of potent, low-molecular weight inhibitors of galectin-3 could provide potent analytical tools for investigating functions of galectin-3 [235,236]. These LacNAc-derivatives could be a challenging platform for shaping new drugs aiming to inhibit galectin-3 effects. As well, new therapies might be developed based on the fact that synthetic peptides bind specifically the CRD of galectin-3 and inhibit metastasis-associated tumor cell adhesion [237]. The most intriguing questions in the field deal with the regulation of galectin-3 expression in different cell types under different physiological and pathophysiological conditions. Despite the fact that some of the signaling pathways have been identified, precise regulatory mechanisms of galectin-3 expression have remained elusive. Since galectin-3 interactions with some signaling molecules have been spotted, it is only reasonable that they may affect particular signal transduction pathways. Once understood, the interplay of these two phenomena, i.e., of the regulation of galectin-3 expression on one hand, and of its biological effects, on the other hand, in a long-term perspective, may help in apprehending the pathogenesis of many diseases and inspire new strategies to fight them.

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