Galectins: Major Signaling Modulators Inside and Outside the Cell

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Abstract: Galectins control cell behavior by acting on different signaling pathways. Most of the biological activities ascribed to these molecules rely upon recognition of extracellular glycoconjugates and establishment of multivalente interactions, which trigger adaptive biological responses. However, galectins are also detected within the cell in different compartments, where their regulatory functions still remain poorly understood. A deeper understanding of the entire galectin signalosome and its impact in cell behavior is therefore essential in order to delineate new strategies to specifically manipulate both galectin expression and function. This review summarizes our current knowledge of the signaling pathways activated by galectins, their glycan dependence and the cellular compartment where they become activated and are biologically relevant.

Keywords: Apoptosis, cell adherence, galectins, immune regulation, oncogenesis, signaling pathways.

1. INTRODUCTION

Galectins (Gals) are proteins with molecular weights ranging from 14 to 35 KDa involved in multiple cellular functions. They are highly conserved throughout evolution [1], suggesting their functional relevance in controlling cell behavior. One of the major properties of galectins is their ability to bind β-galactosides (Galβ1→3,4GlcNAc) through a conserved Carbohydrate Recognition Domain (CRD), independently of the presence of divalent cations. However, there are certain exceptions to this rule, like Gal-10 which binds mannoside-related sugars with higher affinity than β-galactoside binding sugars and Gal-11 which lacks sugar-binding activity [2]. Currently, 15 members have been described, with major differences in their biochemical structures. They have been classified as follows [3-6] (Fig. **1**):

(a) Prototype galectins contain a single CRD and can exist as monomers or homodimers; most of the time, both species are in equilibrium. Gal-1, -2, -5, -7, -10, -11, -13, -14 and -15 belong to this group.

(b) Tandem-repeat galectins contain two non-identical CRDs joined by a short peptide. Gal-4, -6, -8, -9 and -12 are members of this group.

(c) Lastly, the chimera-type Gal-3 contains a single CRD with an extended proline- and glycine-rich N-terminus which promotes oligomerization.

Galectins are soluble proteins that can be found in different cellular compartments such as the nucleus, the cytoplasm and/or the plasma membrane. While extracellular functions of galectins strongly depend on their ability to oligomerize and form supramolecular complexes (often termed lattices) on the cell surface; their intracellular functions do not exclusively depend on such properties [7]. The well known properties of galectins to recognize glycans and glycoconjugates (often they have been discovered for their lectin function) encouraged (and yet encourages) the galectin scientific community to be mostly interested in extracellular galectin functions that are responsible for binding to the extracellular matrix (ECM) and cell surface glycoconjugates, rather than protein-protein or protein-nucleic acid interactions that could be involved in intracellular signaling. In fact, understanding the intracellular functions of galectins is still limited and restricted to some galectin members and therefore this area of research poses a great challenge to gain a complete comprehension of the galectin signalosome.

The complexity of galectins' signaling is even increased due to the fact these lectins are expressed in a variety of tissues (Fig. **1**, adapted from [8]) where they are involved in the regulation of numerous cellular processes [9]. Since galectins have overlapping profiles of tissue expression, it was believed for a long time that they could have compensatory functions.

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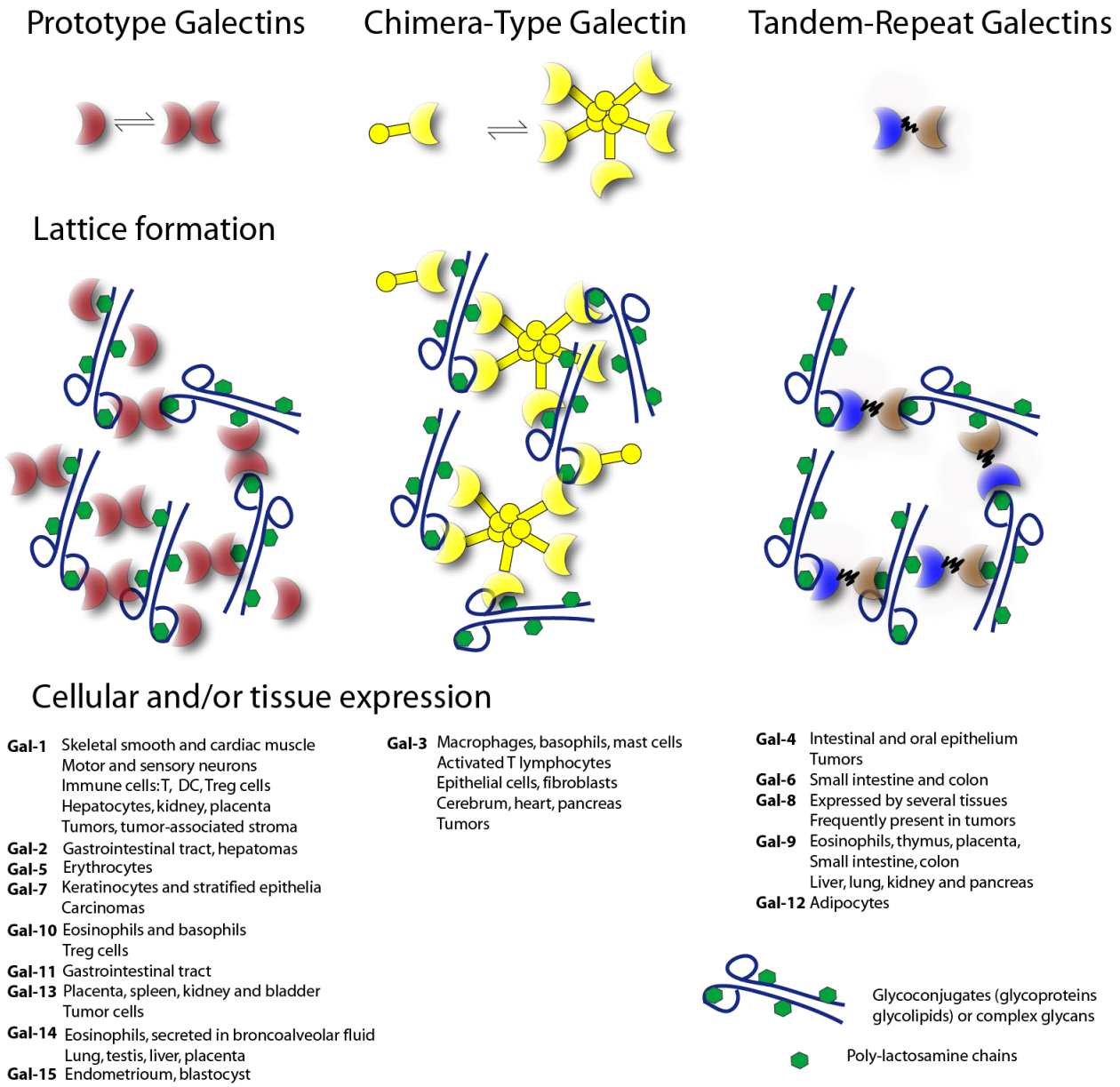

Fig. (1). Classification of Galectins according to their structure. Main Cellular and tissue expression of Galectins.

Current information supports a more complex situation in which each member has particular and non-redundant functions, making them excellent sensors of the cellular microenvironment and endogenous regulators of cell behavior [10].

This review aims to integrate our current knowledge about signaling pathways triggered by galectins at different subcellular localizations that are either glycan-dependent or –independent.

2. INTRACELLULAR GALECTINS

2.1. Factors Affecting Intracellular Localization and Function of Galectins

The cellular localization of galectins depends on several factors:

a) *The proliferative state of the cells under analysis:* upon activation, galectins are immediate-response proteins *via* early gene activation. For instance, following serum stimulation of resting fibroblasts, Gal-3 mRNA expression can be observed 15 to 30 minutes after activation, when cells are still at the G0/early G1 phase of the cycle [11]. While present in both the nucleus and the cytoplasm of proliferating cells, Gal-3 is predominantly cytoplasmic in arrested fibroblasts [12, 13].

b) *Galectin trafficking in the cell*: the major cellular localization of a particular protein does not necessarily imply its exclusion from other sub-cellular compartments. In the case of cytoplasm-nucleus shuttling, the rate of nuclear export could simply exceed that of nuclear import, making this protein more abundant in the

cytoplasm or *viceversa*. The same reasoning might be applied to equilibria between other sub-cellular compartments. It has been demonstrated that intracellular Gal-1 exists in equilibrium between the monomeric and homodimeric state [14, 15]. Meanwhile, Gal-3 exists predominantly as a monomer, with a tendency to render higher molecular weight oligomers [16, 17]. Although these high molecular weight protein complexes could fit into the nuclear pore (exclusion limit of 40 to 60 kDa), experimental evidence suggests that passive diffusion is an unlikely means of translocation between the cytoplasm and nucleus. Elegant experiments using heterodikaryons (fusion of human and murine fibroblasts) where Gal-3 was tracked during its nuclear and/or cytoplasmic trafficking confirmed that the protein certainly shuttles between the nucleus and the cytoplasm [18]. However, identification of the nuclear localization signal (NLS) in Gal-3 has been a major challenge [19-21]. No evaluation of nuclear/cytoplasmic shuttling properties has been performed on other Gals.

It is still unknown whether galectins are constantly produced or stored in a subcellular reservoir. However, galectins are exported to the cell surface through a non-conventional (ER/Golgi-independent) pathway [22]. This unusual route of secretion might prevent the premature binding of galectins to oligosaccharides on nascent glycoproteins. The fine regulatory aspects of this secretion route still remain obscure and merit further efforts to elucidate the molecular mechanisms involved.

c) *Post-translational modifications:* intracellular localization and function of galectins may also be influenced by qualitative parameters. For instance, Gal-3 can be found in a non-phosphorylated form exclusively in the nucleus, while phosphorylated Gal-3 can be found both in the nucleus and cytoplasm [23]. Serine-6 is the major site of phosphorylation of Gal-3, with Serine-12 being a minor site [24]. Casein kinase-I can undertake such phosphorylation function *in vitro* and strongly affects the ability of Gal-3 to bind carbohydrates [25]. Moreover, a Serine-6 mutant Gal-3 that cannot be phosphorylated protects against cellular apoptosis and cell cycle arrest [26]. Others post-translational modifications may have also a role in determining cellular localization of galectins. For instance, intracellular and extracellular spaces differ in their oxidative potential. At this respect, each galectin has particular properties accounting for methods of functional inactivation in particular cellular localizations. For instance, Gal-3 does not appear to be sensitive to oxidative inactivation [27] as it might occur in the case of Gal-1 [28]. In both cases, the amino-terminal ends of Gal-1 and Gal-3 are blocked by acetylation [29].

2.2. Potential Methodological Bias in Studies About Intracellular Localization and Function of Galectins

The information available about the sub-cellular localization of galectins depends on the methods used for galectin detection. For example, saponin and digitonin selectively permeabilize the cholesterol-rich plasma membrane without allowing antibody access to the nucleus [30]. On the other hand, use of more stringent permeabilization agents such as methanol at −20°C or triton X-100 effectively permeabilize the nuclear membrane and allow antibodies to reach nuclear Gal-3 [31, 32].

In addition, attempts to evaluate their endogenous functions have been performed through gain- and loss-of-function experiments. Although these experiments have illustrated some functional aspects of galectins in several systems, some of them merit caution, which is applicable to all galectin members:

i) These kinds of experiments are complex. On one hand, strong compensatory forces are generally observed in loss-of-function systems. On the other hand, gain-of-function models usually imply the induction of protein levels that widely differ from those naturally observed (either under physiological or pathological settings). Indeed, their sub-cellular distribution may not follow natural rules.

ii) As already mentioned with regards to galectin trafficking in the cell, a non-toxic methodology to efficiently block galectin secretion is yet to be developed.

iii) Although we might be able to differentiate the origin of galectins added to the system under study, it is impossible to evaluate the function of these molecules in a particular compartment due to the dynamic nature of the cell. This may thus represent a 'frozen picture' of the cell within a determined time frame, which is quite far from the real situation. Such dynamics are illustrated, for instance, by effects arising from changes in the glycosylation status mediated by an endogenous molecule such as p16INK4a, which regulates the activity of several enzymes involved in the glycosylation pathway and, consequently, has a direct impact on galectin binding to the membrane rafts [33].

2.3. Nuclear Functions of Galectins (Fig. 2)

Several galectins are found in the cell nucleus, where they have been reported to bind to different transcription factors. The list is still incipient and expected to increase as new studies are undertaken. For instance, thyroid-specific transcription factor (TTF-1) and OCA-B are two well known transcription factors that interact with galectins [34, 35]. These interactions are minimally or not at all affected by carbohydrate competition. One exception to this rule is the interaction between galectins and CBP70 (a carbohydrate-binding protein of 70 kDa that can bind to glucose and GlcNAc)

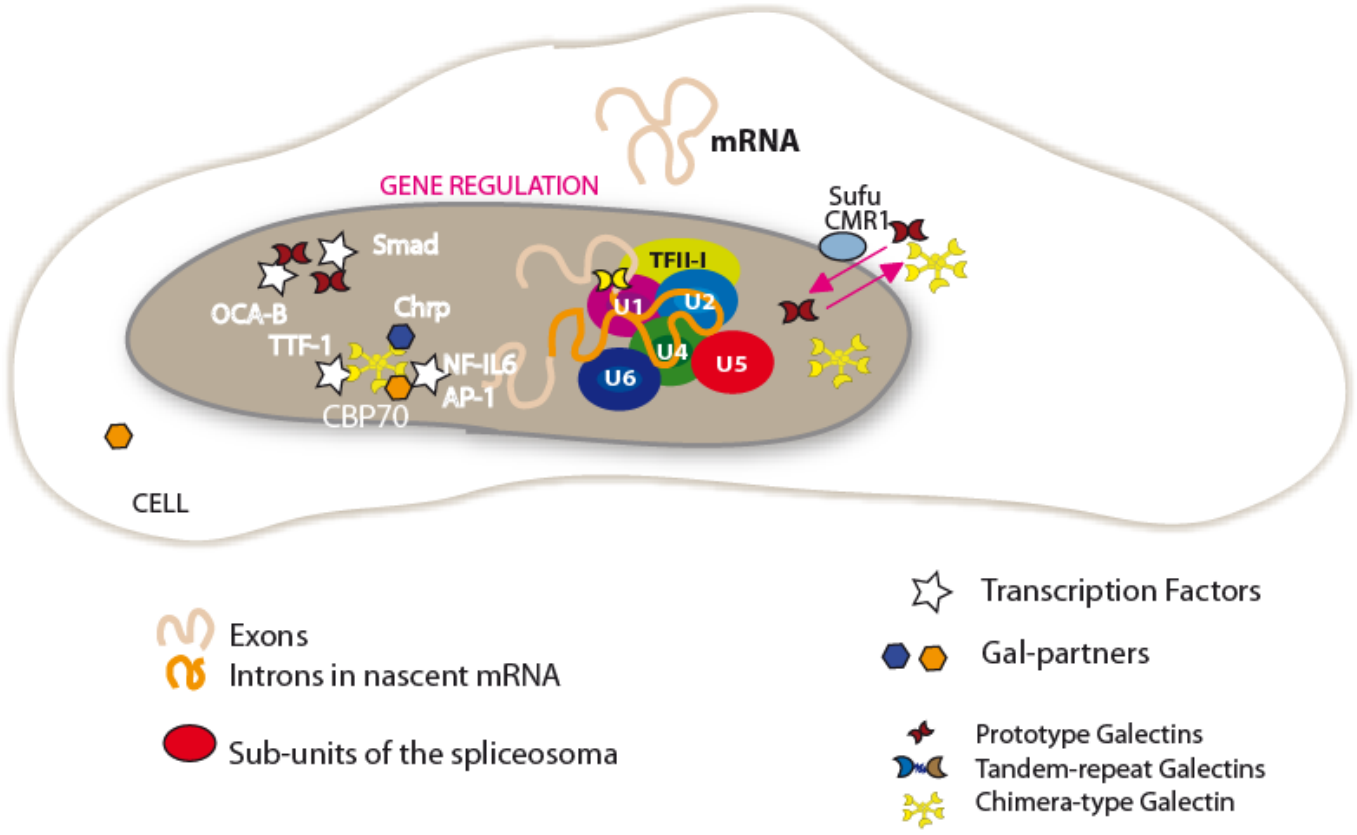

Fig. (2). Nuclear Functions of Galectins.

[36]; binding of CBP70 to its saccharide ligands or to Gal-3 are mutually exclusive.

Galectins enhance and/or stabilize several protein-DNA complexes. Through this type of interaction, Gal-3 binds to multiple *cis*-elements, including the SP1 and CRE sites to activate cyclin D1 promoter activity in human breast epithelial cells [37]. In addition to these specific functions, galectins also play a more structural and general role within intracellular compartments. In fact, not only do Gal-1 and Gal-3 present a diffuse distribution in the nucleoplasm, but also they localize in discrete punctate structures referred to as speckles. Galectins co-localize in some of these speckles with two other components of the splicing machinery: the Sm core polypeptides of snRNPs and the serine- and arginine-rich (SR) family of splicing factors, all found in close association within the nuclear matrix [30, 38, 39]. At the functional level, the speckles correspond to inter-chromatin granule clusters and peri-chromatin fibrils where nascent mRNAs are synthesized and early events of pre-mRNA processing occur [40]. Consistent with this observation, Gal-1 and Gal-3 interact with Gemin4 [39], a member of either the survival of motor neuron protein (SMN) complex or micro-RNA particles such as miRNPs [41, 42]. SMN oligomerizes and forms a stable complex with other proteins, Gemins 2–7, as well as with the core polypeptides of snRNPs [43]. It plays a role in the biogenesis of snRNPs in the cytoplasm before their entry into the nucleus [44]. The importance of Gal-1 and Gal-3 in such associations has been demonstrated by depleting these lectins from nuclear extracts, which causes inhibition of the splicing activity [38, 45-47]. Moreover, the presence of Gal-1 and Gal-3 in the spliceosome seems to be mutually exclusive [48]. This could account for both the normal *in vitro* splicing reactions observed in the absence of only one of these galectins, and the fact that complementation with only one of these galectins is able to recover splicing in a galectin-depleted system [38]. However, the molecular partners of galectins in the spliceosome remain obscure. One promising candidate is the general transcription factor TFII-I [49]. Although Gal-3–TFII-I interaction was identified in nuclear extracts lacking pre-mRNA, it is likely that the same type of interaction occurs within the whole spliceosome structure. All five splicing snRNAs (U1, U2, U4, U5 and U6), but not 5S rRNA, present in nuclear extracts co-precipitate with anti-Gal-3 antibodies [50]. From these potential candidates, a direct interaction between Gal-3 and U1 snRNP has been demonstrated [50]. When isolated, Gal-3-U1 snRNP complexes can bind to exogenous pre-mRNA but not to mature cognate RNA. Antibodies against Gal-3 immunoprecipitate the complete set of U1 snRNAs, snRNP proteins (U1-70K, Sm B/B') and the pre-mRNA with Gal-3 [50]. However, the RNA itself is not a nuclear ligand for Gal-3 [48]. Thus, it is likely that galectins bind pre-mRNA through U1 snRNP, followed by incorporation of other members of the spliceosome complex to further recognize the 5´-splice site and initiate the excision of intron sequences from immature transcripts.

It is important to point out that carbohydrates may influence the association of galectins with nuclear RNPs; lactose pre-treatment of nuclear extracts before performing gradients shifts the distribution of galectins towards the phase of free proteins [50], suggesting that galectins are released from nuclear snRNP complexes following this treatment. This may explain the effect of carbohydrates on pre-mRNA splicing, in which lactose and thiodigalactoside inhibit splicing when added to a complete nuclear extract [45]. However, it should be noted that the carbohydrate-binding property of Gal-1 *per se* is not required for pre-mRNA splicing, as a site-directed mutant (N46D) of Gal-1 devoid of saccharide-binding activity, can still recover splicing activity in a galectin-depleted nuclear extract [49]. Two mechanisms may explain how carbohydrates disrupt these interactions. Firstly, binding sites for glycans and the protein ligand could overlap, leading to saccharide competition inhibiting the binding capacity of the latter. A second proposed mechanism is based on conformational changes induced by the binding of the saccharide to the CRD, which disrupts the interaction between the galectin and other proteins.

The relevance of Gal-1 and Gal-3 in the nucleus is well documented, although other Gals can also be found and have an active role in this cellular compartment. Gal-7 is detected in the nucleus of transformed cells as well [51, 52]. In hepatocytes, Gal-7 shuttles to the nucleus upon treatment with hepatocyte growth factor, where it interacts with phosphorylated Smad3 promoting the nuclear export of this transcriptional co-activator, a process that has a major impact on the expression of TGF-β-responsive genes [52]. Again, this effect is member-specific since it was not observed for other nuclear Gals. Nuclear translocation of endogenous Gal-9 in monocytes is associated with transcriptional activation of pro-inflammatory cytokines. This could be explained, at least in part, by formation of transactivating complexes between Gal-9 with NF-IL6 and/or AP-1 transcription factors [53]. Interestingly, the impact of subcellular localization of Gals is illustrated in this case by opposite (pro-apoptotic) effects observed using exogenous Gal-9 in other cell types [54, 55].

As for the other members of the galectin family, Gal-8 is particularly localized in the nucleus of vascular endothelial cells (ECs) and in the microvasculature of normal and tumoral prostatic and mammary human tissues [56], Gal-10 in human eosinophils [57], Gal-11 in ovine ephitelial cells from the gastrointestinal tract [58], Gal-12 in human adipocytes [59], and Gal-14 in ovine eosinophils [60]. In murine 3T3 fibroblasts and genetically engineered human colon carcinoma cells with stable ectopic expression, Gal-2 was also demonstrated to have a predominant nuclear localization following treatment with different stimuli [61]. Importantly, those nuclear galectins do not seem to co-localize with Gal-1 and Gal-3 and the splicing factor SC35 [61]. Some observations suggest that cellular localization of individual galectins is tightly regulated, and some peculiarities apply for each member. For instance, inverse nuclear/cytoplasm shift has been observed between Gals members in certain pathological situations [62]. This implies that even closely related family members exhibit distinct intra-nuclear localization patterns, eliciting distinct final effects, thus showing a specialization for each member, at least in their nuclear functions.

2.4. Cytoplasmic Functions of Galectins (Fig. 3)

Cytoplasmic expression of galectins has been reported in several studies, serving to a wide range of essential cellular functions.

2.4.1. Galectin-Mediated Signaling in Mitochondria

Mitochondria are organelles where energetic, metabolic and survival processes of the cell take place. In particular, intrinsic and extrinsic signaling pathways of death converge on mitochondrial membrane permeabilization and result in the release of soluble mediators that finally lead to cell death. There is evidence supporting a mitochondrial localization of some galectins, where they play relevant roles as regulators of cell apoptosis.

In human breast carcinoma cells, induction of apoptosis is associated with Gal-3 translocation to the mitochondria where it prevents membrane damage, cytochrome *c* release and the consequent apoptosome activity [63]. This translocation occurs through direct binding of Gal-3 to synexin, a Ca^{2+}- and phospholipid-binding protein. Thus, synexin is a cytoplasmic ligand of Gal-3 related to its apoptosis-inhibiting activity [63]. Upon apoptosis induction in colon tumor cells, Gal-3 co-localize with the ATP synthase inside the mitochondria and inhibits its enzymatic activity [64]. In addition, Gal-3 has a NWGR sequence in its carboxy-terminal, a motif found in the Bcl-2 family of apoptosis repressors [65, 66]. Through this carboxy-terminal end, Gal-3 interacts with Bcl-2 and exhibits anti-apoptotic activity in a lactose-inhibable manner, probably due to CRD proximity to NWRG motif [65]. These apoptosis-related effects of Gal-3 in the mitochondria may play a role in chemotherapy resistance observed in some types of tumors [67].

Gal-7 is another constitutive mitochondrial Bcl-2 interacting partner. In this case, the interaction is independent of the lectin CRD [68]. Upon UV irradiation or stimulation with chemotherapeutic agents, Gal-7 dissociates from Bcl-2, and Gal-7 recovers its pro-apoptotic effect [68]. Altogether, these results indicate that galectins must be regarded as potent regulators of mitochondrial processes associated with cell survival decisions.

2.4.2. Signaling by Cytoplasmic Galectins Controls Essential Cellular Functions

Gal-1 exists in the cytoplasm as both a monomer and a homodimer [14], and there is enough evidence showing that cytoplasmic Gal-1 plays a fundamental role in controlling apoptosis and proliferation-related events [9]. Interestingly, one of the most understood

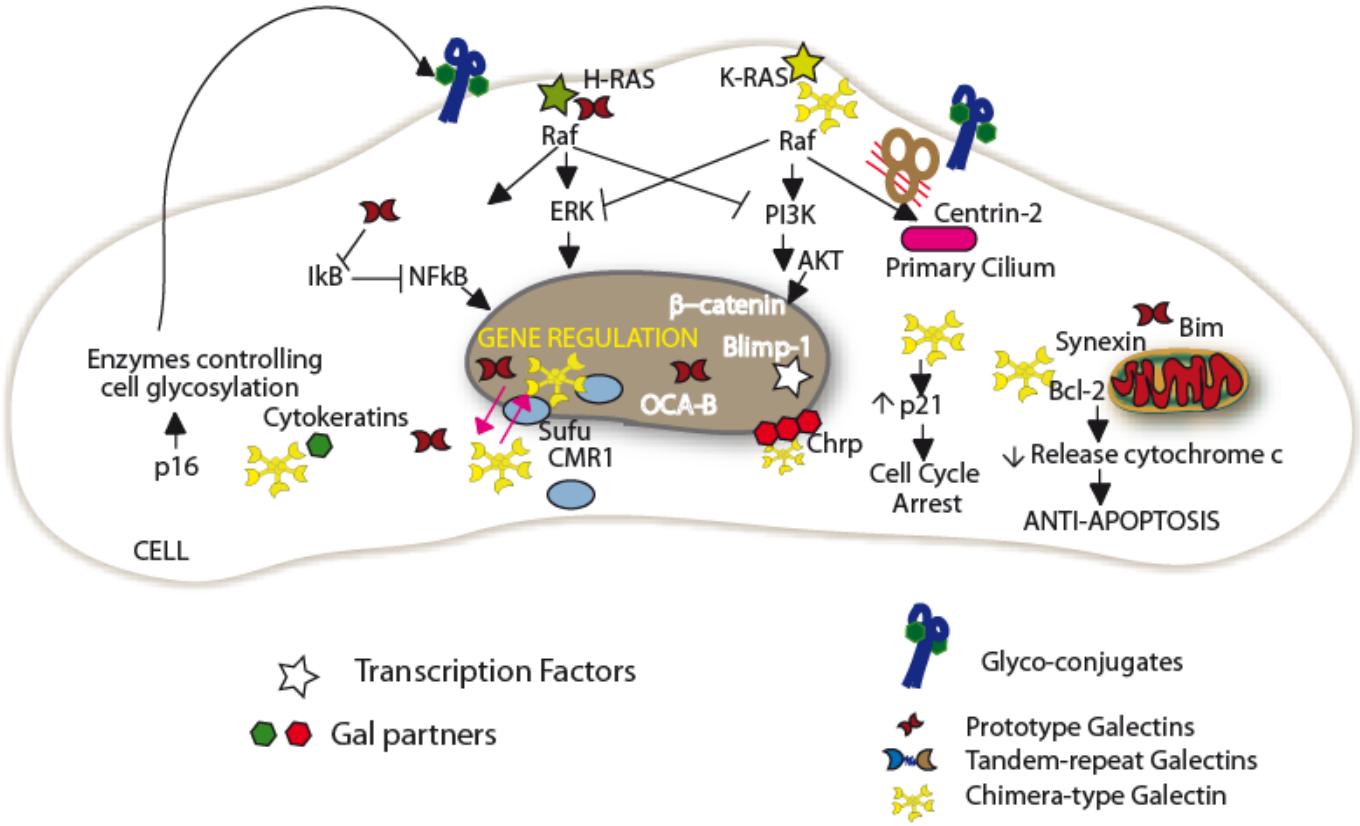

Fig. (3). Cytoplasmic Functions of Galectins.

cellular systems in reference to galectin-mediated signaling refers to immune cells. Gal-1 is expressed at low levels in resting T cells, but is up-regulated during TCR-mediated T cell activation, reaching a plateau in its expression levels during the peak and recovery phases of inflammatory responses [69, 70]. This suggests a critical role of this endogenous lectin in shaping T cell properties. The signal transduction events involved in this process includes recruitment and activation of Lck and Fyn kinases, mitogen-activated protein kinase (MAPK) kinase (MEK)1/extracellular signal-regulated kinase (ERK) and p38 MAPK [69]. In addition, Gal-1 expression regulates the activation of interleukin (IL)-2-dependent signaling of NF-κB transcription factor during the process of T cell activation. In fact, NF-κB translocates to the nucleus, inducing the up-regulation of Gal-1 through functional NFκB binding sites on the promoter of Gal-1 gene, namely *LGALS1*. Moreover, this regulatory control is reciprocal, since exogenous Gal-1 can attenuate NF-κB activation by inhibiting IκB-degradation [71]. These results suggest a regulatory loop between Gal-1 and NF-κB [71] triggered by the p70S6 kinase [69]. Data from studies using Gal-1-deficient mice further support these observations. It was demonstrated that Gal-1-deficient T lymphocytes hyper-proliferate in response to TCR engagement, with a reduction in the levels of apoptosis after activation [72]. Under such conditions, Gal-1 antagonizes ERK and promotes the activity of Lck/Src homology region 2 domain-containing phosphatase 1 (SHP-1). Both regulatory pathways contribute to the pro-apoptotic and negative effects on T cell proliferation [72]. Endogenous up-regulation of Gal-1 is likely to be a homeostatic mechanism to control burst size and function of T cells, actively participating in the contraction phase after T cell activation. Not all T sub-populations are equally sensitive to endogenous Gal-1 deficiency. In fact, Gal-1 functions as an internal negative regulator of Th1 cells; its absence results in a hyper-Th1 response in both *in vitro* and *in vivo* settings [73]. Not only are T lymphocytes sensitive to endogenous Gal-1, but also B cell differentiation and mature B cell function are modulated by this lectin [74]. In fact, Gal-1 is up-regulated in B cells by cross-linking of the BCR and CD40 [75], leading to both positive and negative regulatory effects. On the one hand, Gal-1 and Gal-8 have redundant positive functions in plasma cell formation [76], which depends on the induction of B lymphocyte-induced maturation protein-1 (Blimp-1), the master regulator of plasma cell differentiation [77]. On the other hand, the intracellular association of Gal-1 with the B cell-specific coactivator (OCA-B) negatively regulates BCR signaling [35]. Moreover, intracellular expression of Gal-1 in mature B cells inhibits AKT phosphorylation, leading to Bim up-regulation and, consequently, B cell apoptosis [78]. The subtle differences determining the type of B cell regulation by Gal-1 require further in-depth studies.

Interestingly, molecular effectors activated by Gal-1 are equivalent in a large spectrum of cells, as demonstrated by the similarities between pathways

triggered by this lectin in lymphocytes with those in microglia (previous references and [79]).

Gal-3 also directly affects cell cycle in transformed cells by down-regulating cyclins E and A, and by up-regulating their inhibitory proteins, p21WAF/Cip1 and p27KIP1 [80]. Moreover, this lectin together with p21WAF/Cip1 and PCNA (proliferating cell nuclear antigen; an auxiliary subunit of DNA polymerase δ) are all positively regulated during DNA repair after cell injury with toxins [81]. Additionally, Gal-3 inhibits apoptosis induced by stress [82] through mechanisms that encompass modulation of ERK and AKT activation [83]. It is important to point out that Gal-3 exerts different and sometimes even opposite biological activities according to its sub-cellular localization [84].

Intracellular galectins not only modulate cell survival and proliferation, but also other cellular properties. For instance, Gal-3 has a major role in regulating myeloid cell differentiation [85] and controlling the pro-inflammatory properties of mature macrophages. For instance, during induction of inflammatory responses, macrophages from Gal-3 deficient mice exhibit lower NF-κB activation [86], implying a link between this lectin and inflammation-associated signaling pathways. However, Gal-3 not only controls the classical macrophage activation pathway, the alternative route involving a PI3K-mediated pathway is also regulated by this lectin [87]. This demonstrates the participation of this lectin in the regulation of complex networks of signaling. Gal-3 is also a critical mediator of B cell differentiation and survival. Although Gal-3 is not expressed in resting B cells, its expression is markedly induced after activation with different stimuli, such as IL-4 and CD40 crosslinking, promoting cell survival and regulating the final differentiation of B1 and B2 cells into plasma cells [88, 89]. Gal-3 suppresses apoptosis and promotes differentiation in B cells by down-regulating the Blimp-1 transcription factor after IL-4 stimulation [88].

Two other proteins have been described to interact with cytoplasmic Gal-3. One is Chrp, a protein that is distributed throughout the cytoplasm, but is especially concentrated in a concentric ring at the nuclear envelope [90]. Thus, while the cytoplasm contains both Gal-3 and Chrp, the latter is strikingly excluded from the nucleus where Gal-3 also localyzes. Chrp binds to the carboxy-terminal of Gal-3 and the Gal-3-Chrp complex can bind to carbohydrate-bearing ligands, including laminin [91]. Therefore, the CRD of Gal-3 can simultaneously accommodate two differente ligands, both a carbohydrate and Chrp. Other cytoplasmic partners of Gal-3 are cytokeratins, some of which are glycosylated and, thus, act as natural carbohydrate ligands for cytoplasmic Gal-3 [92]. Altogether, this information highlights the important functions of these small proteins as cellular integrators, whose roles are not only highly dependent on galectin themselves, but rather on the presence and/or conformation of particular partners and the ability to activate one signaling pathway or another. Moreover, they serve as molecular intermediaries activated by membrane receptor ligation and carry extracellular information into the cell.

2.4.3. Role of Endogenous Galectins in Signaling Events Occurring Close to the Inner Leaflet of the Plasma Membrane

Near the cell surface, Gal-1 interacts with the Ras family of GTPases. Interaction of H-Ras with Gal-1 induces a spatio-temporal re-organization of H-Ras into a transient immobilization in short-lived nanoclusters associated to the inner leaftlet of the cell membrane [93]. These H-Ras-Gal-1 complexes require elements of the H-Ras carboxy-terminal hypervariable region and an activated G-domain. Palmitoylation is not required for H-Ras-Gal-1 complex formation, but is needed to anchor H-Ras-Gal-1 complexes to the plasma membrane. The stability of these nanoclusters determines the specificity and magnitude of downstream activation [94]. Gal-1 has a dual role in H-Ras nanoclustering, both as a critical scaffold protein and a molecular chaperone that contributes to H-Ras trafficking by returning depalmitoylated H-Ras to the Golgi complex for re-palmitoylation [95]. Once anchored to the membrane, the H-Ras-Gal-1 complex operates as a molecular switch, inducing the conversion of inactive Ras-GDP into active Ras-GTP, which is then followed by the recruitment of cytosolic effectors such as Raf, and the activation of down-stream extracellular signal-regulated kinase (ERK) and inhibition of phosphatidylinositol-3 kinase (PI3K) activity [96]. Gal-1 is therefore a molecular partner that confers selectivity and strength to the biological effects displayed by H-Ras. The interaction between H-Ras and Gal-1 has a major relevance in the process of H-Ras-mediated cellular transformation. Even though it is independent on glycans [93], it requires a functional CRD, since disruption of a putative prenyl-binding pocket by mutation of leucine 11 to alanine (Gal-1(L11A)) yields a dominant negative protein that negatively regulates the activation of H-Ras by EGF and signaling to ERK, resulting in the inhibition of H-Ras transforming activity [94]. The impact of such interactions is not confined to the tumor cell, since the intercellular transfer of oncogenic H-Ras nanoclusters has been described at the immune synapse between lymphocytes, NK and tumor cells. This kind of transfer of adaptor molecules, called trogocytosis, has significant impact in the modulation of a wide variety of cellular responses [97].

Gal-3 can also interact with Ras proteins. Indeed, this lectin has been observed to act as a selective binding partner and recruiter of K-Ras to membrane nanoclusters [98]. K-Ras nanocluster formation and Gal-3-mediated signaling critically depend on the integrity of the hydrophobic pocket of the Gal-3 CRD, but are independent of glycan binding [98]. Unlike Gal-1, Gal-3 interaction with Ras attenuates ERK but not PI3K activity [98]. Through activation of PI3K, Gal-3 induces AKT phosphorylation, controls glycogen synthase kinase-3β activity. These signaling events have a critical impact on β-catenin levels and its translocation towards the nucleus, where it associates

to TCF4 and controls its transcriptional activity [99]. Such effects of Gal-3 may have major roles in controlling tumorigenesis in several types of cancers [100, 101].

The dynamics of membrane domains are strongly remodeled by Gal-3 in different cell types and biological systems. For instance, early events in the polarization of epithelial cells lacking Gal-3 are characterized by severe perturbations in the microtubular network, which are associated with defects in membrane compartmentalization. Moreover, the absence of Gal-3 impinges on the morphology of the primary cilium; Gal-3 is normally associated with basal bodies and centrosomes [102], where it closely interacts with core proteins such as centrin-2. This association transiently occurs during the process of epithelial polarization. Interestingly, Gal-3-depleted cells contain numerous centrosome-like structures, demonstrating an unexpected function of this protein in the formation and/or stability of centrosomes [102]. The relevance of Gal-3 in this process is illustrated in several polycystic kidney diseases, where the renal collecting ducts are abnormal under Gal-3 deficiency [103]. Moreover, the influence of Gal-3 on membrane dynamics seems to be relevant in different cell types. In physiologic settings, the function of Gal-3 in membrane ruffles and lamellipodia is required for correct migratory properties of dendritic cells [104]. In activated T cells, Gal-3 is primarily located at the peripheral supramolecular activation cluster (pSMAC) and has a destabilizing role at sites of immune synapse, causing down-regulation of T cell receptor (TCR) and subsequent attenuation of early signaling events [105]. These effects require the association with Alix protein, which is involved in protein transport dynamics and the regulation of cell surface expression of certain membrane receptors [105]. Altogether these results highlight the major role that endogenous galectins play in controlling the dynamics of membrane domains and its subsequent signaling events.

3. GALECTIN-INDUCED SIGNALING THROUGH INTERACTIONS WITH GLYCOCONJUGATES ON THE PLASMA MEMBRANE (FIG. 4)

A permissive glycophenotype is the first conditional requirement for efficient signaling triggered by galectins through cell surface glycoconjugates. Therefore, the glycan repertoire and the specificity of galectins will ultimately dictate their functions. For instance, induction of cell death mediated by Gal-1 requires the expression of core-2 *O*-glycan determinants and the reduction of *Sambucus nigra* agglutinin (SNA)-reactive α2-6-linked sialic acid residues on the cell surface [73]. Morevorer, this glycophenotype is highly dependent on the cellular state. In particular, cellular transformation is associated with profound changes in glycan composition [106, 107], which finally determines Gal functions.

A number of galectins (such as Gal-1, -3, -4, -8 and -9) localize in lipid rafts, which are specialized cholesterol-enriched microdomains where galectins can interact with gangliosides, glycosphingolipids and several types of cell surface glycosylated receptors [104, 108-110]. These structures, which include planar and caveolae rafts, promote lattice formation, strengthening the avidity and half-life of ligand/receptor interactions, and organizing centers for molecular signaling. In particular, galectin-glycan lattices have been shown to determine the time of receptor residency by inhibiting the endocytosis of cell surface glycoprotein receptors [111]. *In situ* structural properties of galectins are major determinants of their interactions with glycoconjugates, and the type of signaling elicited. Accordingly, this chapter of the review is organized based on the structural properties of galectins.

3.1. Proto-Type Galectins

Interactions between the glycosylated motifs of several receptors and galectins have been demonstrated to be finely regulated in T cells, thus influencing a diversity of cellular properties including proliferation, survival, differentiation and effector function [7]. Gal-1 interaction with membrane glycoconjugates on T cells induces receptor redistribution and their crosslinking leads to cellular apoptosis in a glycan-dependent manner [112, 113]. The pro-apoptotic effects of Gal-1 contribute to shape peripheral T cell responses but to varying degrees, since not all T lymphocytes are sensitive in the same way [73, 114, 115]. These effects involve activation of caspases-8 and -3, and subsequent cytochrome c release from the mitochondria [116, 117]; activation of acid sphingomyelinase [118]; activation of c-Jun N-terminal kinase (JNK) *via* phosphorylation by PKCδ and PKCθ; activation of mitogen-activated protein kinase kinase 4 (MKK4) and MKK7 converging in upstream JNK activator and enhanced activating protein-1 (AP-1) [116, 119]. However, Gal-1-mediated apoptosis does not require intracellular calcium mobilization [120]. Furthermore, increased Bcl-2 phosphorylation and induction of pro-apoptotic Bad can be elicited by Gal-1 [116, 119]. A second Gal-1-mediated pro-apoptotic pathway that is independent of caspase activation has been reported in T cells and is based on the translocation of mitochondrial endonuclease G without inducing cytochrome *c* release [121]. This pro-apoptotic effect of Gal-1 is counteracted by endogenous Gal-3, demonstrating the close interactions between galectin members in the control of cellular homeostasis [121]. Recently, the use of anti-oxidant-free constructions of Gal-1 confirm the intrinsic pro-apoptotic properties of this particular lectin [122-124]. Additionally, the pro-apoptotic effects of Gal-1 are not only restricted to lymphocytes, as they have also been observed in other cell types [125].

Lastly, events induced by a transient and reversible exposure of phosphatidylserine (PS) following incubation with Gal-1 have also been described in neutrophils. This early event is not followed by mitochondrial changes or nuclear alterations, characteristic apoptosis [126]. Instead, PS exposure

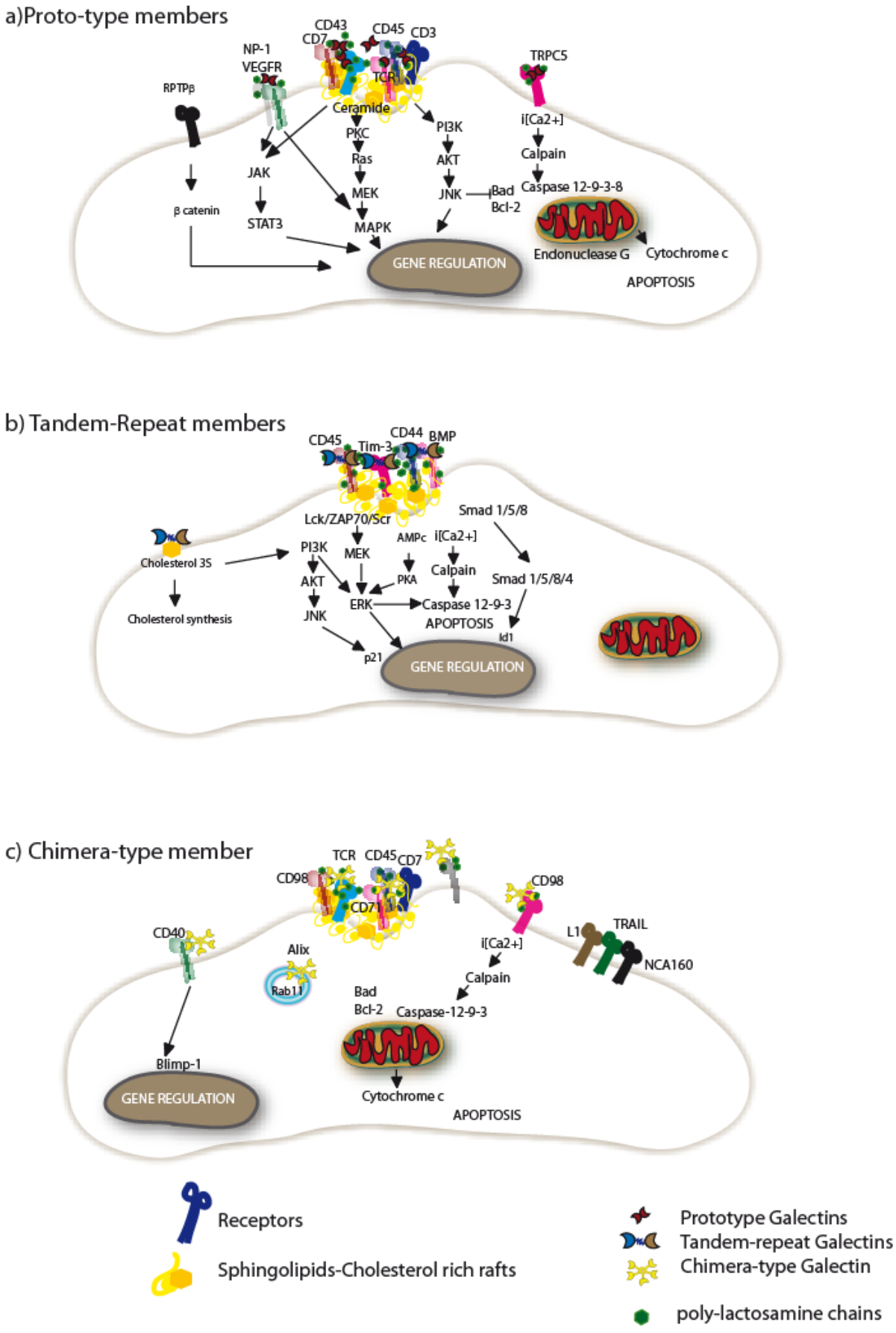

Fig. (4). Interactions of Galectins with glycoconjugates at the cell membrane.

induced by Gal-1 triggers the rapid phagocytic removal of PS-positive cells with important consequences in immune cell homeostasis *in vivo*.

Gal-1 also binds to cell surface glycosylated receptors that are not directly involved in apoptosis induction such as CD2, CD3 and CD4 [120], suggesting a plethora of functions for this galectin, some of which still remain to be elucidated. For instance, T regulatory (Treg) cells ($CD4^+CD25^+Foxp3^+$) express high levels of Gal-1 [127, 128]. Gal-1 expressed at the cell surface of Treg cells may cross-link the ganglioside GM1 on effector T cells inducing a partial and ineffective activation of naïve $CD8^+$ T cells. Interestingly, blockade of Gal-1 on Treg cells did not affect proximal TCR signaling events in $CD8^+$ T cells, such as the activation of CD3ζ, ZAP70 and LAT [129]. On the contrary, Gal-1 interferes with PI3K signaling, blocks ERK/MAPK and Akt signaling [129] and activates the Transient Receptor Potential Channel-5 (TRPC5), a cation channel responsible for Ca^{2+} influx [130], all of which are involved in TCR-induced cell proliferation. Recent evidence supports the ability of Gal-1 to imprint a "Tr1-like" cell immunoregulatory signature, which is defined by IL-10 synthesis, lack of FoxP3 expression, and activation of the c-Maf and aryl hydrocarbon receptor pathways [131]. Other galectins are also essential in controlling Treg function such as the prototype Gal-10 [132] and the tandem-repeat-type Gal-9 [55].

Extracellular Gal-1 is a major regulator of monocyte/macrophage physiology through the activation of MAP kinases. In fact, this molecular signaling influences FcγRI– and MHC-II-dependent functions [133] as well as the chemotaxis of monocytes, but not of macrophages [134]. In addition, exogenous Gal-1 induce CD43/CD45 clustering on the surface of dendritic cells (DC) and leads to the induction of tolerogenic DCs *via* STAT3 phosphorylation. These tolerogenic DC can produce IL-27 and prompt T cells to acquire an IL-10-dependent profile [70]. Gal-1 is expressed by mature DCs and its interaction with the Bam32 adaptor at the synapse zone serves as a key negative regulator of T cell activation [135]. Gal-1 is able to activate additional signaling pathways in DC, inducing calcium influx, engagement of Syk and protein kinase C (PKC), and the activation of upstream signaling pathways [136, 137]. These observations suggest a pivotal influence of Gal-1 on the properties of antigen presenting cells.

Several functional properties assigned to Gal-1 involve its interactions with microenvironmental cells. For instance, Gal-1 is produced by the bone marrow stroma, in particular osteoblasts and reticular cells located on the bone surfaces, and endothelial cells lining sinusoids. It binds to the surrogate light chain (SLC) composed of λ5 and VpreB and with the CD79a/CD79b signaling complex to induce receptor clustering, leading to pre-BII cell proliferation and differentiation [138, 139]. This "developmental B-cell synapse" entails the initiation of intracellular tyrosine kinase activity and pre-BCR signaling [140] inducing CD79a phosphorylation and recruitment of Lyn [141]. In addition, Gal-1 binds to neuropilin-1 (NRP-1) and this axis, together with semaphorine-3A, accounts for some of the immune-suppresive properties displayed by myoblasts and mesenchymal stem cells [142, 143] and offer additional clues to currently unknown signaling pathways triggered through a tolerogenic microenvironment.

Gal-1 can interact with the glycosylated motifs of several receptors expressed on different cell types; the endothelial cell (EC) membrane glycoprotein CD146 is one of such molecular partners. This Gal-1/CD146 interaction controls EC apoptosis program through a glycan-dependent mechanism [144]. Gal-1 also binds to neuropilin-1 (NRP-1) *via* the CRD in ECs, leading to VEGFR-2 phosphorylation and the activation of MAPK and SAPK1/JNK kinases. The global result is modulation of the properties of vascular ECs [145]. In these cells, Gal-1 links hypoxia and neo-angiogenesis, independently of hypoxia-inducible factor (HIF)-1α, HIF-2α and canonical mediators of angiogenesis, but involving reactive oxygen species-dependent activation of NFκB [146, 147]. An additional role of Gal-1 in angiogenesis has been proposed in the placenta, an organ where this lectin is highly expressed. Gal-1 induces the activation of MAP kinases and JAK2 in placental cells, leading to the up-regulation of vascular endothelial growth factor receptor 3 (VEGFR-3) [148, 149]. Furthermore, this lectin is able to counteract the anti-angiogenic effects of the angionex peptide proposed as a therapeutic avenue in several cancers [150]. Altogether, these results highlight the relevance of Gal-1 as a major and non-redundant mediator of cancer associated angiogenesis.

Galectin-2 is an additional prototype member that plays a role in the regulation of T cell behavior; however, it shows some differences with respect to Gal-1. The pro-apoptotic action of Gal-2 entails the activation of caspases-3 and -9, enhanced cytochrome c release, disruption of the mitochondrial membrane potential, and an increased Bax/Bcl-2 ratio [151]. The subtle signaling differences observed among individual members of the galectin family may account for the peculiarities associated with each galectin, even when sharing a similar structure.

3.2. Tandem-Repeat Galectins

Galectin-9 can induce apoptosis in many cell types, mostly by intracellular calcium mobilization and activation of a calpain-caspase-1 pathway [152]. In Th1 cells, this signaling pathway is activated by Gal-9 binding to the glycoprotein Tim-3 in a CRD-dependent manner [54]. On the other hand, differentiation of Th17 cells involves Gal-9, but not Tim-3 [153]. Lattice formation is a requisite for Gal-9-mediated death since constructions containing only a single CRD do not have any pro-apoptotic effect [154].

Exogenous Gal-9 promotes maturation of DC, inducing more efficient Th1 immune responses. This effect does not depend on the CRD of the lectin, as a Gal-9 mutant lacking β-galactoside-binding activity

retains its immunostimulatory properties. DC maturation is mediated by the activation of p38 MAPK and ERK1/2 kinases [155]. However, up-regulation of endogenous Gal-9 in $CD4^+CD25^+$ Treg cells substantially contributes to the suppressive activity of these cells [156, 157], demonstrating clear opposing effects for the same lectin according to its cellular source.

In human umbilical vein endothelial cells (HUVECs), engagement of Toll-like receptor (TLR)3 elicits the expression of Gal-9 *via* PI3K and retinoic acid-inducible gene-I (RIG-I) [158]; this probably constitutes a relevant response towards viral stimuli. In osteoblasts, Gal-9 is present in lipid rafts, where it interacts with CD44 and bone morphogenetic protein (BMP) to induce the phosphorylation of Smads and consequently promote osteoblast differentiation [159]. Gal-9 also induces c-Src/ERK signaling, an additional molecular control associated with osteoblast proliferation [109]. These Gal-9-mediated events of osteoblast differentiation and proliferation are not triggered by other members of the galectin family, further demonstrating the subtle differences in galectin activity in each cell type. In myeloma cells, Gal-9 activates JNK and p38 MAPK signaling pathways, resulting in apoptosis through the activation of caspase-8, -9, and -3 [160].

Glycosylated motifs of CD44 also bind Gal-8, which closely interacts with ECM proteins in order to regulate the pro-apoptotic properties of this galectin member in activated synovial leukocytes [161]. The biological effect of Gal-8 is mediated at least partially by JNK phosphorylation [161]. Activation of JNK has a direct inhibitory effect on the cell cycle through increased p21 accumulation [162]. Recently, CD166 [activated leukocyte cell adhesion molecule (ALCAM)] was identified as a specific Gal-8-binding partner in normal vascular ECs [56], signaling pathway whose relevance requires further investigation.

Gal-8 has a dual effect in peripheral T cells, where it is able to co-stimulate naïve T cells and elicit apoptosis when these cells become activated [163]. The co-stimulatory effect, which is inhibited by thiodigalactoside, is associated with CD45, whose phosphatase activity lowers the TCR activation threshold by Lck dephosphorylation and induces the following conventional TCR downstream signal transducers: ZAP70, Src, PI3K, PKC and MAPK [164]. Remarkably, T cell co-stimulation does not require the tandem-repeat structure of Gal-8 [163]. On the other hand, Gal-8 can induce apoptosis of immature $CD4^{high}CD8^{high}$ thymocytes through a caspase-dependent pathway [165]. In addition, Gal-8 has a pro-apoptotic effect on Jurkat cells that involves phospholipase D/phosphatidic acid signaling, which enhances the activity of type 4 phosphodiesterases (PDE4). Gal-8-induced PDE4 activation leads to a strong ERK1/2 activation and consequently, expression of the death factor Fas ligand and caspase-mediated apoptosis [166]. These antagonic effects of Gal-8 on T lymphocytes are a clear example of the functional complexity of this protein family.

Gal-4 is expressed in the epithelium of the gastrointestinal tract and participates in the fine control of intracellular cholesterol levels. The bivalent structure of Gal-4 is important for its binding to 3-*O*-sulfated Gal residues and to cholesterol 3-sulfate present at the cell surface. Surprisingly, the latter ligand does not have a β-galactoside moiety [167]. This characteristic of Gal-4 is unique within the galectin family [168] and may represent an additional regulatory mode of this family of proteins. In fact, cholesterol 3-sulfate is an important component of cell membranes with regulatory roles in the activities of several high-impact enzymes (serine proteases, protein kinase C isoforms and phosphatidylinositol 3-kinase) [169]. The intracellular exchange between cholesterol and cholesterol 3-sulfate could represent a regulatory checkpoint of cholesterol levels since cholesterol 3-sulfate inhibits cholesterol esterification and modulates hydroxymethylglutaryl-CoA reductase, which is the rate limiting enzyme for cholesterol synthesis [170]. Apart from its role in modulating cholesterol levels, Gal-4 expression at intercellular junctions correlates with the establishment of a polarized epithelium in the esophagus, consistent with the role played by this lectin in bridging cells together [171]. In addition, Gal-4 expressed by intestinal epithelial cells bind to the CD3 molecule on the lamina propria T cells, inducing biological effects that require further research. On the one hand, Gal-4/CD3 interaction promotes $CD4^+$T cell activation through PKCΦ [172]. On the other hand, this interaction can have the opposite effect; secreted Gal-4 induces apoptosis in activated peripheral and mucosal lamina propria T cells *via* calpain, but not caspases [173]. This trait is shared with Gal-9, but not with the other pro-apoptotic galectins, providing more evidence that despite common binding partners on T cells, each galectin member activates different pathways to trigger T cell death.

3.3. Chimera-Type Galectins

Galectin-3 is a family member that displays both pro- and anti-apoptotic activities, according to its subcellular localization. Cytoplasmic Gal-3 protects cells from apoptosis by preserving mitochondrial membrane integrity and antagonizing cytochrome *c* release [63, 174]. On the other hand, exogenous Gal-3 exerts a negative regulatory effect on T cell activation. Induction of T cell apoptosis is one of the cellular mechanisms responsible for this negative regulation [175]. While both extracellular Gals-1 and -3 induce apoptosis on T lymphocytes, they do so using different mechanisms. Whereas Gal-1 induces apoptosis of double-negative and double-positive human thymocytes with equal efficiency, Gal-3 preferentially acts on double-negative thymocytes [176]. These differences could be explained, at least in part, by different galectin-binding patterns. Accordingly, it has been demonstrated that CD45, CD71 and CD7 are involved in Gal-3-induced T cell death [175, 176].

Following Gal-3 binding, CD45 remains uniformly distributed on the cell surface, in contrast to CD45 clustering induced by Gal-1 [176]. By interacting with these cell surface glycosylated receptors, extracellular Gal-3 triggers apoptosis *via* caspase-3 activation and significant cytochrome *c* release. These molecular events are elicited in response to a carbohydrate recognition process, since lactose inhibits these pro-apoptotic effects [175]. Gal-3 is also a binding partner of CD98, a protein implicated in the regulation of cell adhesion, growth and apoptosis [177]. The cytoplasmic domain of CD98 can bind to the cytoplasmic tail of *β*-integrin inside the cell, promoting integrin activation. This suggests that Gal-3-CD98 can modify integrin activation in T cells [177] and also regulate intracellular Ca^{2+} concentration in Jurkat cells [178].

A second mechanism responsible for the negative effects of extracellular Gal-3 on T cells is its ability to bind to the TCR complex. Gal-3 can form a multivalent lattice with glycoproteins of the TCR-CD3 complex on T cell surfaces, thereby restraining the lateral mobility of the TCR complex [179]. β1,6N-acetylglucosaminyl-transferase V (*Mgat5*) knockout mice have been particularly useful in understanding the functional nature of the galectin lattice and the associated phenotypes *in vivo*. *Mgat5*$^{-/-}$ T cells display a reduced threshold for activation due to reduced Gal-3 binding to the TCR. Indeed, as Gal-3 binding normally prevents precocious TCR clustering, the mutant mice thus display spontaneous autoimmunity [179].

Gal-3 is abundantly expressed in myeloid cells and plays a pivotal role in controlling glycan-dependent and independent endocytosis [180]. Galectins are endocytosed in a raft-dependent and clathrin-independent manner, which has a fundamental impact on the membrane half-life of several receptors [181]. Once endocytosed, Gal-3 remains as a functional protein for a long time in Rab11^{+} acidic endosomes, where it may have important endogenous roles [182]. For instance, Gal-3-containing endosomes attenuate EGFR endocytosis through interaction with Alix [183], a component of the multivesicular endosomes required for correct membrane dynamics [184].

This lectin binds specifically to the neuronal adhesive glycosylated proteins L1 and N-CAM and myelin-associated glycoprotein MAG [185]. Additionally, Gal-3 plays an important role in the growth and stability of polarized epithelial cells, which are characterized by distinct apical and basolateral membrane domains that are separated by tight junctions. Gal-3 mediates non-raft-dependent glycoprotein targeting to the apical membrane [186], where the lectin is secreted and interacts with ECM proteins to contribute to apical membrane organization [187]. These observations argue in favor of the multiple regulatory functions Gal-3 has in a plethora of cell types.

Gal-3 expression is much higher in many malignant cells than in corresponding normal cells. Thus, this galectin is expected to be secreted more by the former. Tumor-secreted extracellular Gal-3 may have apoptosis regulatory functions on transformed cells and/or an inactivating effect on cancer infiltrating T cells, thereby facilitating tumor evasion of immune responses. Gal-3 binds to different receptors in transformed cells [188]. In metastatic colon adenocarcinoma cells, cell surface Gal-3 can interact with TNF-related apoptosis-inducing ligand (TRAIL), impeding the trafficking of death receptors by anchoring them to glycan nano-clusters, thereby blocking the execution of the apoptosis signal [189]. In LNCaP prostate cancer cells, exogenous expression of Gal-3 inhibits anticancer drug–induced apoptosis through the down-regulation of Bad, as well as the inhibition of cytochrome c release and caspase-3 activation [190]. In lymphoma B cells Gal-3 interacts with CD45, regulating its phosphatase activity [191]. Depletion of Gal-3 sensitizes these tumor cells to chemotherapeutic-induced apoptosis [191]. Altogether, these observations highlight the anti-apoptotic properties of exogenous Gal-3 in transformed cells.

4. GALECTIN-MEDIATED SIGNALING THROUGH INTERACTIONS WITH EXTRACELLULAR MATRIX COMPONENTS AND INTEGRINS (FIG. 5)

As this field has been previously reviewed [9, 177], this report will only discuss controversial aspects related to this kind of interactions. The ECM constitutes essential physical scaffolding for cellular constituents and provides crucial biochemical and biomechanical cues that are required for tissue morphogenesis, differentiation and homeostasis. Galectins interact with ECM glycoproteins (laminin [192, 193]; fibronectin [192, 194] or tenascin [185]), activating important signaling cascades that have profound impacts on cell adhesion and motility due to a complete reorganization of the actin cytoskeleton and formation of focal adhesion plaques [195, 196]. Interestingly, this kind of interactions could lead to opposite cellular effects; galectin engagement promotes cell attachment in some cell types whereas in others it results in cell detachment, spreading and migration [162, 196-201].

Those opposite effects of galectins on cell adhesion may be explained by several parameters: first, they depend on the concentration of the lectin present in the media and/or its cellular localization [202], thus being highly influenced by both intrinsic and microenvironmental factors. Second, they also depend on the valency and biological properties of galectins, the relevance of this concept is highlighted by the fact that galectin-mediated signaling is extremely dependent on the state of the cell. For instance, Gal-3 does not affect melanoma cell attachment unless it is treated with transglutaminase [203], which can efficiently oligomerize Gal-3 [204]. As a rule, monovalent ligand binding is expected for many prototypic galectins, and dimeric prototypic or tandem-repeat galectins when these are present in large excess relative to the cell surface or matrix molecules. On the other hand, under conditions of equal amounts of galectin and ligand, the interaction might stimulate adhesion by crosslinking cell surface and matrix molecules. The threshold concentration required to switch

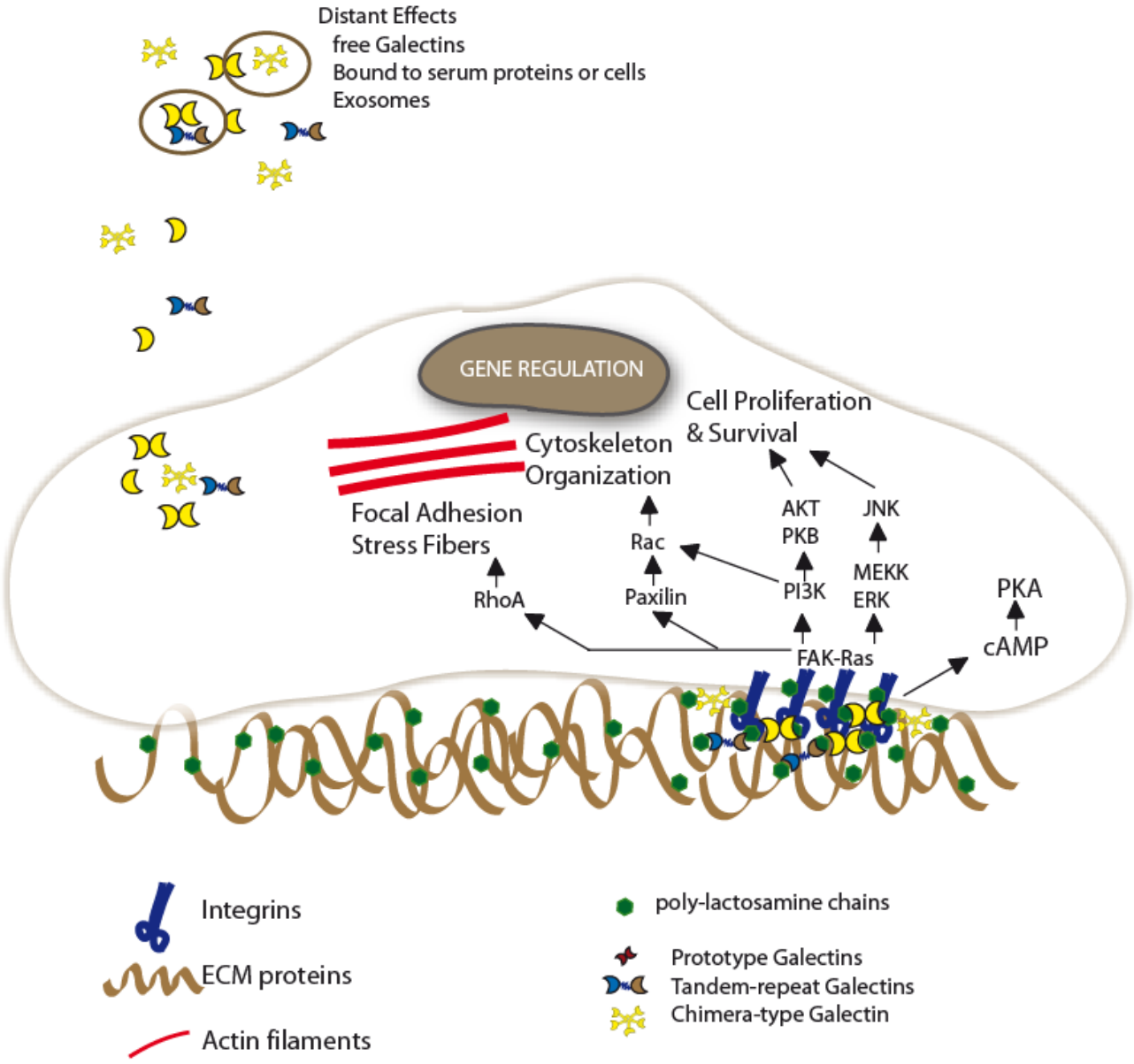

Fig. (5). Extracellular functions of Galectins.

Gal-3 from an inhibitory to a stimulatory modulator of cell adhesion to a laminin substrate was determined to be on the range of micromolar [205]. This concentration is probably exceeded in the cytoplasm of many cells [171] and may even be higher in the secretory vesicles that deposit the lectin in the extracellular space. In addition and as previously discussed in *chapter 2.1* other post-transcriptional modifications of galectins confer alterations in their extracellular properties. Finaly, a third parameter that affects galectin/integrin/ECM protein interactions during cell attachment is the direct activation of integrins. Subtle differences in galectin affinity for specific glycans may induce different lattice conformations and thus may influence spatial interactions with integrins [206] and, as consequence may have an impact on functional outcomes.

Last but not least important, protein/glycan interactions in the ECM serve as a repository for cytokines and molecules that determine cell polarization and tissue organization. Tumor cells obtained from *Mgat5*-deficient mice fail to undergo epithelial-to-mesenchymal transition (EMT) and are insensitive to multiple cytokines, including epidermal growth factor, fibroblast growth factor, platelet-derived growth factor, insulin growth factor 1 and transforming growth factor [207]. Moreover, galectin interactions that are relevant in normal homeostatic processes are also implicated in pathology. For instance, interaction of fibronectin and laminin with Gals-3 and -8 are associated with an increased metastatic potential of transformed cells [208].

5. PARACRINE AND SYSTEMIC EFFECTS OF GALECTINS (FIG. 5)

Galectins may not only play autocrine functional roles. They can exert biological effects at distant sites, inducing activation of signaling cascades in cells that

express the appropriate and permissive glycophenotype. Epithelial cells localize Gal-3 towards apical domains of the membrane, ready to be secreted to elicit particular biological functions [182]. It was demonstrated that Gal-1 derived from primary tumors can have specific effects on distant metastases [209]. Moreover, Gals-3 and -9 are present in exosomes [210, 211], which are vesicles derived from normal and tumors cells. Through this transport system, tumors could have an impact on the processing and presentation of tumoral antigens in draining lymph nodes. Alternatively, galectins can be transported bound to serum proteins [212, 213] or circulating cells such as platelets [214].

6. RELEVANCE OF GALECTINS IN HUMAN DISEASES

Galectins have been implicated in several biological processes associated to human diseases ([215], and Table **1**). For instance, higher levels of galectins have been observed in the circulation of patients with different pathological conditions [212, 216]. Therefore, it is proposed that galectins in serum or other biological fluids could be used as diagnostic and/or prognostic biomarkers for certain diseases. However galectins can not only serve as biomarkers, they could also be interesting targets for new therapies proposed in several pathological conditions. Understanding the "galectin signalosome" is therefore essential to delineate rational therapeutic strategies based on the specific control of galectin expression and function.

7. CONCLUDING REMARKS

Galectins can trigger and/or modulate a broad variety of cellular signaling pathways at particular locations where they are confined (intracellularly, at the cell surface, extracellularly *via* interactions with the ECM and even at remote sites). Gal-1 and Gal-3 are the best studied members of the galectin family, particularly with regards to their exogenous glycan-dependent functions. However, further studies are required to dissect the complete galectin signalosome. This review integrates our current knowledge about signaling pathways triggered by galectins at different subcellular localizations that are either glycan-dependent or –independent (summarized in Table **2**).

Extracellularly, galectins are responsible for translating exogenous changes occuring at the cellular glycome into the activation or inhibition of endogenous cell signaling pathways that ultimately induce defined biological effects. Although galectin binding to β-galactosides has a relatively low affinity (at the μM range) [217], two parameters should be taken into account to fully understand the major impact of these glycan-binding proteins on cell behavior. First, glycan-galectin interactions can promote the cross-linking of

Table 1. Relevance of Galectins in human pathologies.

Disease		Galectin	Galectins' Role in Pathology	Ref.
Atheroesclerosis		Gal-1	Adhesion, migration and proliferation of vascular SMC Bind to lipoprotein (a)	reviewed in [224]
		Gal-3	Involved in ethiology of plaque foam cells. Secreted by foam cells	
Diabetes		Gal-1	Inflammation and immune regulation, other mechanisms?	[225]
		Gal-3		[226]
Other autoimmune diseases		Gal-1 Gal-2 Gal-3	Survival and/or differentiation of particular immune cells Regulation of cytokine profile	reviewed in [2]
Heart failure		Gal-3	Mediator of fibrosis and cardiac remodeling	[227-229]
Pregnancy-associated diseases		Gal-1	Vascular remodeling, immune dysfunctions, other mechanisms?	[230-232]
		Gal-13		[233, 234]
Cancer	Colorectal cancer	Gal-1	Transformation, cell adhesion, angiogenesis and immune-escape	[235]
		Gal-3		[236, 237]
		Gal-4		[238]
	Thyroid cancer	Gal-3		[239, 240]
	Prostate cancer	Gal-1		[146, 241]
		Gal-3		[146, 241, 242]
		Gal-8		[146]
	Breast cancer	Gal-1		[209, 243]
		Gal-8		[212]
	Hematological malignancies	Gal-1		reviewed in [244]
		Gal-3		

Table 2. Galectins' interactoma and its carbohydrate dependence.

Sub-Cellular Localization	Galectin	Binding Partner	Function	Carbohydrate Sensitivity	Refs
Nucleus	Gal-1	OCA-B	Transcription	No	[35]
		Protein/nucleid acid complexes	RNA splicing	No	[30, 48]
	Gal-3	TTF1	Transcription	No	[34]
		CBP70	?	Yes	[36]
		Protein/nucleic acid complexes	Transcription	No	[37]
			RNA splicing	No	[38, 39, 48-50]
	Gal-7	Smad-3	Transcription	?	[52]
	Gal-9	NF-IL6	transcription	?	[53]
Mitochondria	Gal-3	Synexin	Apoptosis	?	[63]
		ATP synthase	Apoptosis	?	[64]
		Bcl-2	Apoptosis	Yes	[65, 66]
	Gal-7	Bcl-2	Apoptosis	No	[68]
Cytoplasm	Gal-1	Ras	Signaling	No	[93]
	Gal-3	Chrp	?	No	[91]
		Cytokeratins	Cytoskeleton structure	Yes	[92]
		Ras	Signaling	No	[98]
		Alix	Protein transport dynamics	?	[105]
Cell membrane	Gal-1	Glycosylated Receptors (CD2, CD3, CD7, CD43, CD45, Thy1)	Microdomains/signaling/apoptosis	Yes	reviewed in [245]
		Neuropilin-1	Migration/Adhesion	Yes	[142, 143, 145]
		Glycolipids (GM1, GT1b, GD1a, GD1b, GM3)	Immunosuppression	?	[130]
	Gal-3	EGFR/TGFbR	Proliferation/Migration	Yes	[207, 183]
		CD45, CD71 and CD7	Microdomains/signaling/apoptosis	Yes	[175, 176]
		CD98	Cell adhesion, growth and apoptosis	Yes	[177]
	Gal-4	Cholesterol regulation	Metabolism	No	[168]
		CD3	Pro-inflammatory cytokines	?	[172]
	Gal-8	CD44	Apoptosis	Yes	[161]
		CD45	Proliferation	Yes	[164]
	Gal-9	CD44	Cell differentiation	Yes	[159]
		Tim-3	Apoptosis	Yes	[54]
Extracellular matrix	Gal-1	β1 Integrin	Adhesion/Proliferation/Apoptosis	Yes	reviewed in [245]
		Fibronectin/Laminin/Tenascin	Cell Migration/Spreading	Yes	[194]
	Gal-3	β1 Integrin	Adhesion/Proliferation/Apoptosis	Yes	reviewed in [245]
		Fibronectin/Laminin/Tenascin	Cell Migration/Spreading	Yes	[192, 193]

glycoconjugates, their re-distribution or segregation at the cellular membrane thus increasing the avidity and half life of these interactions [218]. Second, since glycans are one of the most abundantly expressed molecules in the cell, these interactions are highly frequent. This second point should be considered in the context of the dynamics of glycan fluctuations, since protein glycosylation notably changes according to the cellular state (*e.g.* differentiation, activation, etc). In particular, tumor cell transformation is associated with glycosylation profiles that are substantially different from their normal counterparts [219]. Particularly, cellular transformation is associated with the increased expression of β-1,6-GlcNAc branched N-glycans, which favors galectin binding [220]. Altogether, these observations suggest an important role of galectin-glycan interactions in the control of cellular behavior.

However, lattice formation is not the only parameter determining specificity of signaling. Even in the case of members sharing structural similarities, each individual

galectin has highly specialized and finely-tuned cellular functions. Moreover, the same galectin member can trigger even opposite effects [84, 162, 163, 175]. This can be explained by variations in the saccharide specificity and glycan availability for galectins [2, 217, 221, 222]. This might provide a rational explanation for the functional divergences displayed by these multifunctional glycan-binding proteins. In this regard, the activation of a particular pathway not only depends on the expression of a given receptor, but also on its degree and quality of glycan modification [223]. Subtle differences in receptor glycosylation can be induced through modulation of particular glycosyltransferases and glycosidases, whose global activities rely on the plasticity of the cell in response to endogenous and microenvironmental signals. This level of regulation adds a high degree of complexity which dictates the activation of particular pathways. Yet, it is not only the expression but also the activity of glycan-modifying enzymes which determines the resulting glycome of the cell at a given time. Some additional specialization could be due to changes in the localization of the galectins and their molecular partners (bound to cells or other proteins, at the ECM, in membrane lipid rafts or located within the intra-cellular space), their dynamics (fluidity inside such compartments) and their structural properties (monovalent or expressed in highly order structures); all these are instrumental to modulate galectin binding and signaling. Thus, understanding galectin functions may not be as simple as originally predicted and further analysis should take the above mentioned parameters into consideration to define the precise role of each given galectin in particular biological processes.

Interestingly, most of the signaling pathways triggered by galectins converge towards few common effector molecules including MAPK, JNK, JAK-STATs, mitochondrial adaptors and transcription factors. Under such circumstances, how might signaling processes elicited by galectins account for the high degree of diversity in biological functions? Different alternatives could provide rational explanations for these divergent effects even under converging signaling.

The simpliest explanation holds the view that individual members of the galectin family can transduce different signaling events *via* subtle or large variations in their interactive properties. Moreover, the activation of a given signaling pathway by galectins not only depends on its level of expression, but also on the properties of the molecular partners they bind. In this respect, intracellular galectins regulate the recruitment and activity of some adaptors, kinases and transcription factors, each of which contribute to the modulation of signaling cascades. Conformational changes in both galectins and their molecular partners could account for diversity, modifying their binding and signaling properties. Post-translational modifications (such as oxidation, cleavage, phosphorylation, acetylation and others) can also have an important effect on their interacting properties and endogenous functions. This issue is particularly relevant given the sensitivity of some galectins to their inactivation/functional changes under the particular conditions prevalent in subcellular microenvironments. This type of regulation adds an increasing level of biological complexity that greatly influences the overall signaling effect, since the resulting outocome is not defined by the *per se* expression of individual molecules and their potential ability to interact with each other, but also by the qualitative modifications occurring inside the cell at a given time. Additionally, cross-talk between simultaneously activated signaling pathways each triggered at different intensities and/or involving alternative intermediate adaptors, may affect downstream responses.

The compartamentalization effect should also be taken into account as cellular dynamics may restrict molecular interactions to particular cellular localizations. For instance, following internalization of galectin-cell surface glycosylated receptor complexes, the recovery of these internalized materials could lead to additional intracytoplasmic function whether appropiate intracellular membrane dynamics exist. Merging these concepts will lead to a better understanding of how the galectin signalosome controls cell behavior and poses a major challenge to this scientific field.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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