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Journal of Cellular Signaling Review Article

Galectin 3 and Glial Cells of the CNS: A Fruitful Crosstalk with Remyelinating Potential

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Received date: July 12, 2020, **Accepted date:** August 28, 2020

Abstract

Galectin-3 (Gal-3), the only chimera-like galectin, has three structural domains: (a) the NH2 terminal domain containing serine phosphorylation, important for nuclear localization, secretion and oligomerization; (b) a sequence susceptible to metalloprotease (MMP) cleavage; and (c) a C-terminal domain containing the carbohydrate recognition domain (CRD) and an anti-death motif. In turn, oligodendrocytes (OLG) are the resident cells responsible for CNS myelination. OLG undergo morphological and molecular changes along several maturational stages. In this context, the present review summarizes current knowledge of Gal-3 role in OLG differentiation, myelination and remyelination in experimental models of multiple sclerosis (MS). Recombinant Gal-3 (rGal-3) accelerates both OLG differentiation, evidenced by an increase in the number of mature cells to the detriment of immature ones, and actin cytoskeleton dynamics. These changes respond to rGal-3 influence on Akt, Erk 1/2, and β-catenin signaling pathways. Our most recent results reveal a key temporal window spanning OPC and pre-OLG states for this pro-differentiating action of rGal-3 and identify several targets for rGal-3 binding including proteins related to the cytoskeleton, signaling pathways, metabolism and intracellular trafficking. Gal-3 expression in microglial cells during CPZ-induced demyelination and upon the onset of remyelination favors an M2 phenotype, hence fostering myelin debris phagocytosis through TREM-2b phagocytic receptor and MMP activity modulation, leading to OLG differentiation and remyelination. This evidence indicates that Gal-3 mediates the glial crosstalk and thus fosters remyelination both by driving OLG differentiation and promoting a phagocytic microglial phenotype. These studies also shed light on some of the mechanisms underlying Gal-3 action and open doors for the identification of new Gal-3-regulated pathways to control OLG proliferation and differentiation. Altogether, these data unveil the therapeutic potential of Gal-3 in demyelinating diseases such as MS and may allow the development of new targets.

Keywords: Galectin-3, Oligodendrocytes, Myelination, Remyelination, Microglia, Cytoskeleton, Cuprizone

Introduction

Galectins (Gals) are a group of 15 proteins characterized by a highly conserved carbohydrate-recognition domain (CRD) and made up of approximately 130 amino-acids which bind β-galactose in glycoconjugates. Gals are classified into three groups according to their structures [1-3], i.e. proto, chimera and tandem-repeat. Proto Gals, which have a single CRD, include Gal-1, Gal-2, Gal-5, Gal-7, Gal-10, Gal-11, Gal-13, Gal-14, and Gal-15. In turn, tandem-repeat Gals contain two similar CRD and comprise Gal-4, Gal-6, Gal-8, Gal-9, and Gal-12. The only member of the chimera class, Gal-3 has three structural domains: (a) the NH2 terminal domain containing serine phosphorylation, important for nuclear localization, secretion and oligomerization; (b) a sequence susceptible to metalloprotease (MMP) cleavage; and (c) a C-terminal domain containing the CRD and an anti-death motif [4,5]. Worth pointing out, the N-terminal domain allows the formation of pentamers upon the interaction of Gal-3 monomers with glycoproteins or glycolipids.

Gal-3 has been associated to physiological processes including immunomodulation and cell proliferation, adhesion, migration, growth and differentiation [6,7], all largely determined by Gal-3 cellular localization, specific tissue, or specific pathological condition. Gal-3 can be found in the nucleus, on the cell surface, in the

extracellular space [8,9] and, depending on the cell type, also in exosomes or microvesicles [10-12] Given that Gal-3 lacks a signal sequence which may guide its translocation to the endoplasmic reticulum and further enable the classical secretory pathway, the secretion of Gal-3 proceeds in a non-classical fashion [9].

Galectin-3 in the Nervous System

Gal-3 has shown neuronal and glial expression [13]. The nerve growth factor (NGF) mediates Gal-3 expression in mouse dorsal root ganglia (DRG) neurons and macrophage-like cells, which suggests a role in the promotion of neurite outgrowth, neural cell adhesion [14,15], and neurite stabilization in the cerebellum [16]. Gal-3 is expressed in inflammatory cells, including astrocytes, microglia, macrophages, dendritic cells, eosinophils, mast cells, NK cells, and activated T and B cells. Increasing evidence has recently suggested a dual modulatory role for Gal-3 in neuroinflammation and neurodegeneration [17,18]. Gal-3 promotes microglia phagocytic capacity and chemotactic properties, thus amplifying the immune response by binding to the CCR receptor [19,20]. Upon activation, Gal-3-expressing microglia undertake myelin debris phagocytosis through FcgR, CR3/MAC-1 and SRAI/II receptors and foster remyelination upon injury or disease [21]. Gal-3 has been also recently shown to activate phagocytosis by targeting the cytoskeleton twice: first, by advancing cofilin activation, enabling filopodia and lamellipodia to extend and engulf myelin debris; and second, by advancing actin/myosin-based contraction through K-Ras.GTP/PI3K signaling, hence causing filopodia and lamellipodia to retract and internalize myelin debris [22].

Oligodendroglial biology

Oligodendrocytes (OLG) are resident central nervous system (CNS) cells in charge of myelination, i.e. the physiological process through which axons are insulated and thus provided with metabolic and trophic support for rapid saltatory conduction of action potentials [23]. Starting from a highly proliferative PDGFRα- and NG2-positive bipolar cells, oligodendroglial progenitors (OPC) later become pre-OLG, more ramified cells expressing CNPase, Olig 1 and O4, to finally develop into mature OLG (m-OLG) with MBP, APC and PLP expression and the ability to form myelin membranes [24,25].

Although Gal-3 mRNA expression has not yet been evaluated in OLG, Gal-3 has been immunocytochemically detected in rat primary cultures of OPC and m-OLG, with higher levels in the latter [26]. This Gal-3 appears to be cleaved in OPC by MMP2 but stabilized in m-OLG, which suggests variations in its biological activity during OLG differentiation [26]. Recombinant Gal-3 (rGAl-3) treatment in OPC promotes dose-dependent OLG differentiation [26], which may respond to secretion by Gal-3-expressing microglia during normal oligodendrogenesis [26,27]. In agreement, treatment with conditioned media from wild type (WT) microglia, but not Gal-3 knockout (*LGALS*$^{-/-}$) microglia, promotes OLG differentiation. These results are tightly associated to the glycoconjugates present in OPC cell surfaces, as glycosylation signature analysis has shown that only OPC possess a permissive glycophenotype expressing the carbohydrates required for Gal-3 binding [26].

The participation of cytoskeleton dynamics in oligodendroglial differentiation and myelination has been proposed as a two-stage model of actin dynamics: first, pro-polymerizing actin cytoskeleton dynamics promote OPC branching up to full OLG maturity; second, the actin cytoskeleton shifts to depolymerizing dynamics, which triggers myelination. These stages are partly regulated by the relative levels of MBP and actin disassembly proteins colfilin-1 and gelsolin, sequestered and inactivated by phosphatidylinositol 4, 5-bisphosphate (PIP2) in the plasma membrane. MBP then competes for PIP2 binding with cofilin-1 and gelsolin and displaces them to initiate the disassembly of actin filaments in m-OLG [28]. In turn, rGal-3 has been shown to accelerate the differentiation of purified OPC, as evidenced by an early increase in m-OLG markers and a decrease in immature OLG ones [29]. Worth highlighting, these results were accompanied by the acceleration of actin dynamics in the two-stage model described above, as evidenced by an earlier polymerization peak and subsequent depolymerization. In parallel, these studies revealed an increase in Akt activation and β-catenin, MBP and gelsolin levels, together with a decrease in Erk1/2 activation [29]. Along these lines, recent reports have shown that the Akt/mTORC and Erk1/2 pathways play independent and cooperative roles in OLG differentiation along development and adulthood both *in vitro* and *in vivo* [30], and that Erk 1/2 inhibition favors OLG generation and recovery in demyelinating diseases [31].

In this context, studies using a single pulse of rGal-3 at different stages of oligodendroglial maturation were conducted to identify the temporal window of rGal-3 action and unravel its direct targets promoting differentiation. Results showed that rGal-3 promotes OLG differentiation at OPC and pre-OLG stages, generating an increase in pAkt, β-catenin, and F-actin [32]. The phosphorylation of substrate p4EB-P1 also indicated that mTORC1 is activated by rGal-3 treatment, which contributes to MBP expression and OLG maturation [33]. However, rGal-3 treatment at the m-OLG stage failed to increase MBP expression, F-actin and β-catenin levels, or Akt activation, and produced lesser mTORC1 signaling activation. Strikingly, results also revealed an increase in gelsolin and a decrease in pErk 1/2 at all stages, both indicative of

OLG differentiation [34]. Taken together, these interesting results suggest that the action of rGal-3 depends on the repertoire of glycolipids and glycoproteins present at the time of treatment.

Proteins directly interacting with rGal-3 at OPC and pre-OLG stages

Given rGal-3 pro-differentiating action at OPC and pre-OLG stages, studies were further carried out on direct rGal-3-molecule interaction at these two stages through co-immunoprecipitation and subsequent identification by mass spectrometry [32]. At the OPC stage, interacting molecules connected with cell proliferation included casein kinase II subunit alpha (CKII), proliferating cell nuclear antigen (PCNA) and voltage-dependent anion-selective channel protein 1 (VDAC1), which, through rGal-3 inhibition or activation, may limit proliferation to induce OLG differentiation [35-37]. As for cytoskeletal proteins, rGal-3 was observed to interact with ras-related C3 botulinum toxin substrate 1 (Rac1) and its inhibitor, rho GDP-dissociation inhibitor 1 (RhoGDI), which may support rGal-3-dependent activation of Rac1 [29]. Most importantly, direct interaction was detected between rGal-3 and gelsolin, which is consistent with cytoplasmic gelsolin expression in OPC differentiation and maturation and suggests that cytoskeleton severing proteins may be crucial for morphological changes required for OLG differentiation [34]. In terms of energy metabolism and lipids, these assays provided evidence of rGal-3 interaction with transaldolase (TAL) and ATP-citrate synthase, which are involved in the production of the large amount of lipids necessary for myelin synthesis and protection from the deleterious effects of free radicals [38]. Regarding signaling pathways, rGal-3 was found to interact with carboxypeptidase E (CPE), which modulates the Wnt-β-catenin pathway and β-catenin levels, and with poly-ADP-ribosepolymerase 1 (PARP1), whose inhibition in the cuprizone (CPZ) model and in MS type III lesions leads to an attenuation of demyelination by increasing Akt activity [39] and favoring the oligodendroglial lineage in the subventricular zone (SVZ) [40]. Finally, on the subject of intracellular trafficking, rGal-3 interacted with Rab-5A, which may be involved in rGal-3 internalization and redistribution in different subcellular compartments.

In contrast to the OPC stage, no proteins involved in proliferation were found to interact with rGal-3 at the pre-OLG stage. Among cytoskeletal proteins, rGal-3 was found to interact with JUP, Rac1, Arp3, gelsolin, F-actin-capping protein subunit alpha-1 (CAPZA1), actin depolymerizing protein, and septin-11 (SEPT11) and septin-7 (SEPT7), cytoskeletal GTPases promoting the formation of actin filaments. rGal-3 also interacted with microtubule-related proteins such as cytoplasmic dynein 1 light intermediate chain 2 (DYNC1LI2) and microtubule-actin cross-linking factor 1 (MACF1), with calmodulin (CAM), involved in MBP-actin interaction [41], and with stathmin (STMN1), which promotes OLG branching [33]. Taken together, these interactions emphasize the key role played by rGal-3 in the regulation of actin filament dynamics. In turn, signaling pathways interacting with rGal-3 included nucleoside diphosphate kinase A (NME1), whose function is to inhibit OPC differentiation [42] and, strikingly, many subunits of the proteasome, which is known to degrade β-catenin, profilin 1, gelsolin, and MBP in OLG [43-45]. This suggests an inhibitory relationship between rGal-3 and these subunits of the proteasome, which may explain the increase in the levels of several proteins found upon rGal-3 treatment. Furthermore, previous studies from our laboratory indicate that inhibition of the proteasome leads to OLG differentiation and promotes remyelination in the CPZ model [46,47]. Interestingly, rGal-3 also interacted with inositol monophosphatase 1 (IMPA1), involved in the supply of inositol for phosphoinositide synthesis, which plays a crucial role in the dynamics of MBP-gelsolin-F-actin. Of note, rGal-3 was also found to interact with Erk2, which may explain the decrease in Erk activation induced by rGal-3 treatment, perhaps by blocking the phosphorylation site or generating conformational changes which prevent its activation. Interaction was also found with protein quaking, which regulates the splicing, export and stability of mRNA and protein translation, and is necessary for MBP stability and correct actin polymerization in OLG [48]. Last, regarding intracellular traffic, rGal-3 again interacted with Rab proteins. Altogether, this evidence suggests that extracellular rGal-3 is internalized and then redistributed together with target molecules to the corresponding subcellular compartments where it mediates cytoskeleton dynamics, proliferation, lipid synthesis, and signaling pathways necessary to drive OLG differentiation.

Myelination

Electron microscopy morphometric analysis have revealed a critical role for Gal-3 during myelination, reflected by *LGALS3*$^{-/-}$mice hypomyelination, lesser myelin integrity and abnormal compaction, associated with substantial behavioral alterations [26]. To elucidate the role of Gal-3 in myelination *in vivo*, studies by our group focused on Gal-3 expression at postnatal day 5 (P5), P10, P15 and P20 using transgenic mice expressing the enhanced green fluorescent protein (EGFP) driven by the promoter of oligodendroglial protein CNPase (CNP–EGFP). Gal-3 expression showed substantial changes during white matter development, with high expression levels at P5 and a reduction along myelination. In agreement, high levels of Gal-3 were found at P5 in microglial cells localized in the corpus callosum (CC) and cingulum, fairly close to CNPase

cells. Microglia appear in developing fiber tracts prior to the onset of myelination, and the transient generation of the bulk of OLG in white matter coincides with the presence of amoeboid microglia in the CC. Evidence available suggests that this ameboid microglia may have a role not only in clearing a path for axons, but also in clearing cell debris during gliogenesis. In addition, a recent study using microglia depletion has revealed an essential role for postnatal microglia in proper OPC and OLG development and homeostasis [49]. Gal-3 colocalizes with CNPase cells with mature-OLG-like morphology and has been detected at low levels in astrocytes at P10 and P15, and at high levels in the SVZ at all ages evaluated. The absence of Gal-3 affects the migration of neuroblasts from the SVZ through the rostral migratory stream toward the olfactory bulb [50] by a mechanism implying increased phosphorylation and activation of epidermal growth factor receptor (EGFR). Gal-3 also promotes the proliferation of cultured neural progenitors and its inhibition decreases the proliferative response of the SVZ after brain ischemia [51]. Our group has also proven Gal-3 to promote cell commitment toward the oligodendroglial lineage in neurosphere cultures [26]. However, Gal-3 overexpression with electroporation in the SVZ induces no inflammation in healthy postnatal gliogenesis, with a larger percentage of striatal astrocytes but a smaller percentage of OLG [52].

Gal-3 in MS and its experimental models

Oligodendroglial injury leads to demyelination, which is followed by the formation of new myelin sheaths as a regenerative response –a process referred to as remyelination [53]. This process has been described in animal models and in human demyelinating diseases such as MS [54-56]. MS disease progression varies considerably among patients, although the most frequent clinical presentation involves recurring symptoms followed by total or partial recovery, namely classic relapsing-remitting MS. Symptoms become progressive in around 50% of untreated patients and lead to clinical deterioration for several years, a stage identified as secondary progressive MS. However, about 15% of MS patients present relentless disease progression as from onset, which constitutes primary progressive MS [57-59]. The progressive stage partly responds to incomplete remyelination, which produces the loss of axonal metabolic support and concomitant axonal and neural degeneration, leading to progressive disability [60,61].

The endogenous and exogenous roles of Gals in glial cells upon demyelination/remyelination have been thoroughly reviewed recently by Jong et al. [62]. Serum from patients with secondary progressive MS has been recently shown to contain auto-antibodies against Gal-3, which may be responsible for blood brain barrier (BBB) progressive damage [63]. Actually, membrane-bound Gal-3 in human brain microvascular endothelial cells is a target for auto-antibodies present in secondary progressive MS serum [64]. In addition, OPC treated with cerebrospinal fluid (CSF) from patients with primary progressive MS present a significantly more ramified morphology than control or relapsing remitting MS CSF, and a pro-differentiating transcriptome: downregulation of PDGFRα and LINGO1 genes and upregulation of MAG and, interestingly, LGALS3 genes, which establishes a link between Gal-3 upregulation and increased OPC branching. Gal-3 upregulation has also been observed in post-mortem brain tissue of patients with primary progressive MS [65]. These findings indicate that Gal-3 is a positive target for OLG differentiation in MS. However, the presence of anti-Gal-3 auto-antibodies is responsible for BBB damage, a negative effect which might be counteracted through neutralizing therapy.

Although experimental demyelination models fail to fully replicate the complexity and heterogeneity of MS, they have rendered fruitful results and allowed the development of various treatments. Several well-established experimental demyelination models include those mediated by immunity, viruses, and toxins. The most widely used animal model of CNS demyelination, experimental autoimmune encephalomyelitis (EAE) consists in the immunization of mice with myelin oligodendroglial glycoprotein. *LGALS3*$^{-/-}$mice display clearly diminished CNS macrophage infiltration during EAE, which leads to lower production of pro-inflammatory cytokines interleukin IL-17 and IFN-γ and lesser disease severity [18]. Gal-3 is also involved in IL-4-mediated macrophage alternative polarization, and its effect in EAE may be thus attributed to microglial activation and proliferation [66,18]. An increase in Gal-3 expression has been reported in macrophages and microglia in the CNS of EAE mice [67], which was then reduced by copolymer 1 through an increase in antigen-specific Th2 response and secretion of IL-10, and a diminished production of pro-inflammatory cytokines and Gal-3.

Gal-3 is induced in several cell types involved in damaged axon and cell debris removal and axon regeneration and remyelination, which hints at a neuroprotective role of Gal-3 in EAE mice [68]. In addition, Gal-3 key role in the phagocytosis of disrupted myelin has been also reported in Wallerian degeneration, as evidenced by increased Gal-3 expression in myelin phagocytic microglia and its absence in non-myelin phagocytic ones [22,69,70]. Lack of Gal–3 actually accelerates Wallerian degeneration by modifying toll-like receptor and pro-inflammatory cytokine expression in the injured sciatic nerve [71].

MS-induced inflammation may decrease SVZ cell proliferation and thus hinder repair. Gal-3 expression

increases in active human MS lesions [72], in periventricular regions in human MS and after a virus-induced MS model, in which the loss of Gal-3 restores SVZ proliferation through a reduction in the number of immune cells [73].

Another useful demyelination model, CPZ administration produces massive demyelination by mature oligodendroglial apoptosis through pathogenic T cell-independent mechanisms. This model keeps the BBB intact [74,75] and is characterized by astroglial activation and resident microglial recruitment, whereas peripheral macrophage infiltration remains controversial [76-81]. Phagocytosis of myelin debris by microglia in CPZ demyelination, once again key to the onset of remyelination [82], is concomitant with an increase in phagocytic receptor TREM-2b expression and CD200R and TNF-α production [83].

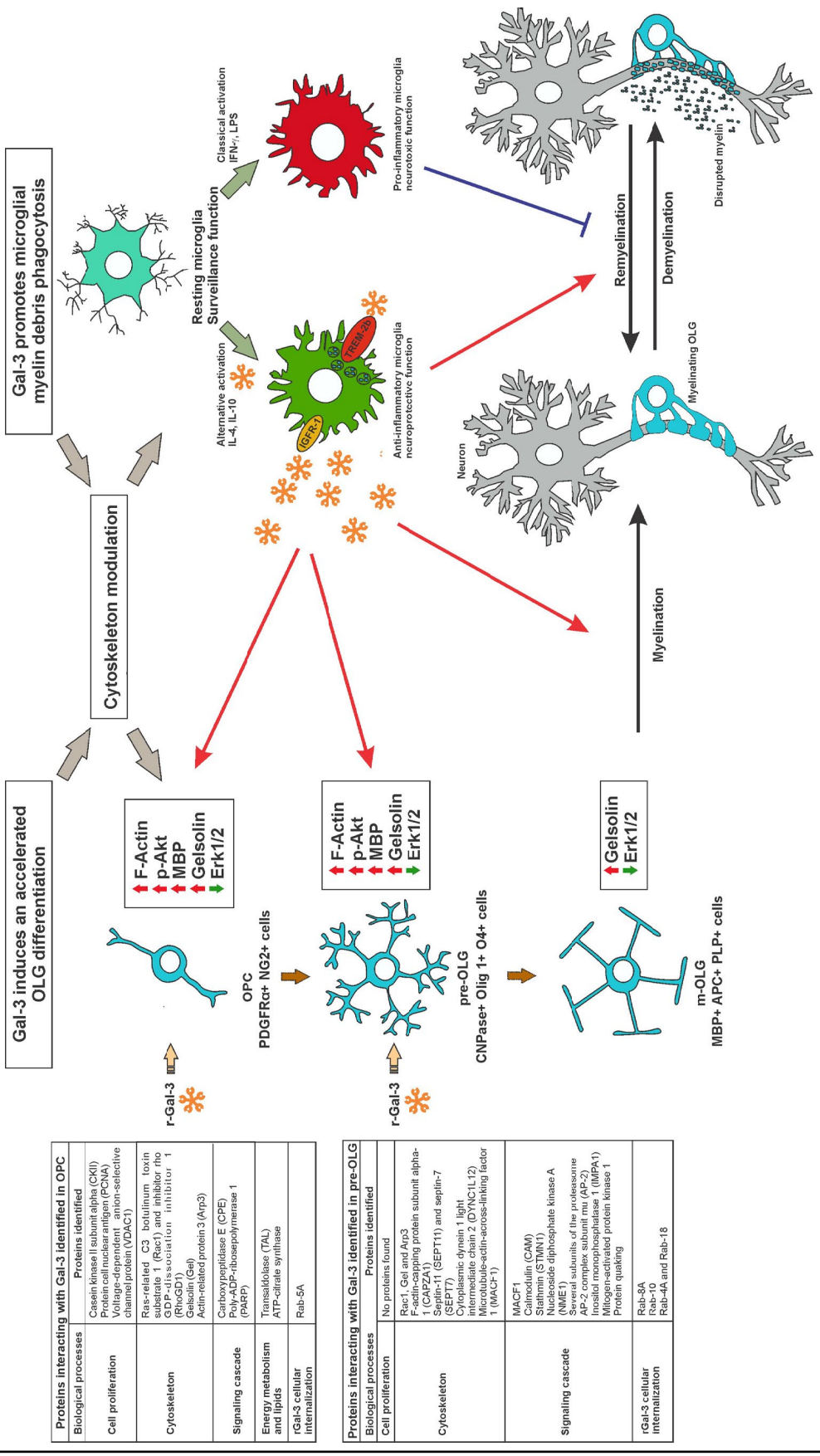
Studies by our group using the CPZ model to determine Gal-3 participation in demyelination/remyelination [84] rendered comparable courses of demyelination in both *LGALS3*$^{-/-}$ and WT mice up to the 5th week of treatment. However, while WT mice initiated spontaneous remyelination in the 5th week of CPZ treatment, when the CPZ diet was still in place, *LGALS3*$^{-/-}$ mice underwent constant demyelination up to the 6th week, with pronounced astroglial activation. Studies of Gal-3 expression in WT mice showed its upregulation in microglia but not in astrocytes. Interestingly, only WT mice displayed activated microglia with ED1 (CD68) expression and TREM-2b upregulation during CPZ-induced demyelination, while CPZ-treated *LGALS*$^{-/-}$ mice exhibited more numerous microglia with activated caspase-3, which suggests that the absence of Gal-3 alters the microglial response against demyelination. M2-cell-conditioned medium has been shown to enhance OLG differentiation *in vitro*, and M2 cell depletion has been proven to impair OLG differentiation *in vivo*, which indicates that M2 cell polarization is a key factor for efficient remyelination [85]. Therefore, microglial Gal-3 expression may hence favor the onset of remyelination, either by inducing an M2 phenotype or exerting a direct effect on OLG differentiation.

Gal-3 and MMP modulation in remyelination

MMP-2 and MMP-9 catalyze the cleavage of Gal-3 into a 22 kDa fragment which has a CRD and a 9 kDa polypeptide containing the N-terminal domain [86,87]. This process changes the properties of the 22 kDa fragment relative to the function and binding properties of the CRD [87]. Although the relationship between Gal-3 and MMP has been mostly studied in connection with tumorigenesis and metastasis [88], MMP-9 deletion in nervous tissues has been shown to protect against ischemic brain injury, diminishing neuroinflammation and preserving BBB integrity [89]. In turn, *LGALS*$^{-/-}$ mice submitted to hypoxic brain injury show lower expression of MMP-9, while WT mice co-express Gal-3 and MMP-9 in activated microglia [90]. MMP can also induce myelin protein degradation *in vitro* [91-93], and are involved in postnatal myelination, myelin maintenance, and remyelination [93,94,95]. Also, MMP-3 can mediate mature OLG apoptosis and microglial activation, with the production of microglial inflammatory cytokines and the consequent exacerbation of neural cell degeneration [96]. Interestingly, WT mice show an increase in MMP-3 expression and a decrease in CD45, TNFα, and TREM-2b+ cells during remyelination, while *LGALS*$^{-/-}$ mice exhibit no changes either in demyelination or remyelination [97].

Conclusions

Gal-3 is essential for microglial polarization following CNS injury, although with different effects on different injuries probably responding to time- and context-dependent factors [98]. Some possible molecular mechanisms underlying Gal-3 neuroprotection from microglia are particularly interesting in OLG biology, myelination and remyelination processes. Oligomerized Gal-3 molecules may crosslink to IGFR upon binding to their glycans on the surface and delay their removal by endocytosis, which results in prolonged microglial mitogenic signaling [99,100]. This is particularly relevant because IGF-1 acts directly on OLG differentiation and myelination [101] and, when secreted by microglia, reduces OLG apoptosis in the CPZ model [102]. Moreover, Gal-3 involvement in alternative microglial polarization induced by IL-4, as that taking place in macrophages [103], may also explain the effect of Gal-3 on cell commitment toward the oligodendroglial lineage, as IL-4-activated microglia favor oligodendrogenesis through a mechanism mediated by IGF-I, while IFN-gamma-activated microglia favor neurogenesis [104]. Taken together, our results have shown that Gal-3 expression in microglial cells during CPZ-induced demyelination and upon the onset of remyelination favors an M2 phenotype, hence fostering myelin debris phagocytosis through TREM-2b phagocytic receptor and MMP activity modulation, leading to OLG differentiation and remyelination [84,97] (Figure 1). Given that unsuccessful remyelination may result from inefficient removal of myelin debris by microglia, unveiling the mechanisms controlling phagocytosis may prove an effective approach to reversing disability and curbing disease progression. In addition, Gal-3 has been recently identified as a novel endogenous TREM-2b ligand [105]. Most interestingly, Gal-3 has been lately shown to activate microglial phagocytosis through a mechanism involving cytoskeleton modulation [22]. In line with these studies and emphasizing the key role played by Gal-3 in the regulation of actin filament dynamics, our most recently published results showed that rGal-3 accelerates oligodendroglial



Proteins interacting with Gal-3 identified in OPC	
Biological processes	Proteins identified
Cell proliferation	Casein kinase II subunit alpha (CKII) Proliferating cell nuclear antigen (PCNA) Voltage-dependent anion-selective channel protein (VDAC1)
Cytoskeleton	Ras-related C3 botulinum toxin substrate 1 (Rac1) and inhibitor rho GDP-dissociation inhibitor 1 (RhoGD1) Gelsolin (Gel) Actin-related protein 3 (Arp3)
Signaling cascade	Carboxypeptidase E (CPE) Poly-ADP-ribosepolymerase 1 (PARP)
Energy metabolism and lipids	Transaldolase (TAL) ATP-citrate synthase
rGal-3 cellular internalization	Rab-5A

Proteins interacting with Gal-3 identified in pre-OLG	
Biological processes	Proteins identified
Cell proliferation	No proteins found
Cytoskeleton	Rac1, Gel and Arp3 F-actin-capping protein subunit alpha-1 (CAPZA1) Septin-11 (SEPT11) and septin-7 (SEPT7) Cytoplasmic dynein 1 light intermediate chain 2 (DYNC1L12) Microtubule-actin-across-linking factor 1 (MACF-1)
Signaling cascade	MACF1 Calmodulin (CAM) Stathmin (STMN1) Nucleoside diphosphate kinase A (NME1) Several subunits of the proteasome AP-2 complex subunit mu (AP-2) Inositol monophosphatase 1 (IMPA1) Mitogen-activated protein kinase 1 Protein quaking
rGal-3 cellular internalization	Rab-8A Rab-10 Rab-4A and Rab-18

Figure 1: Extracellular Gal-3 accelerates oligodendrocyte (OLG) differentiation and promotes microglia myelin debris phagocytosis through cytoskeleton modulation, thus favoring remyelination. rGal-3 induces OLG differentiation at oligodendroglial progenitor cell (OPC) and pre-OLG stages, generating an increase in pAkt, MBP and F-actin. While rGal-3 treatment at the m-OLG stage fails to increase MBP expression, F-actin or Akt activation, an increase in gelsolin and a decrease in pErk 1/2 are observed at all stages. The main proteins found to interact with recombinant Gal-3 (rGal-3), obtained through co-immunoprecipitation and mass spectrometry analysis of OPC and pre-OLG lysates by Thomas and Pasquini 2019 (32), include proteins related to cell proliferation, cytoskeleton, signaling cascades, lipids, and those probably mediating rGal-3 cellular internalization. APC, adenomatous polyposis coli; CNPase, 2´, 3´-cyclic-nucleotide 3´-phosphodiesterase; MBP, myelin basic protein; NG2, neural/glial antigen 2; Olig, oligodendrocyte transcription factor; PDGFRα, platelet-derived growth factor receptor α; PLP, myelin proteolipid protein; mature OLG (m-OLG); interleukin-4 (IL-4), interleukin-10 (IL-10), interferón γ (IFN-γ), lipopolysaccharides (LPS).

differentiation also through cytoskeleton modulation [29], which was strongly supported by the identification of Gal-3 direct interaction with a key protein in actin dynamics control such as gelsolin [32] (Figure 1). Given that Gal-3 binds to multiple targets, its effects on OLG biology and myelination may reflect direct and indirect actions, i.e. those mediated by microglia, astrocytes or peripheral cells. Nowadays, numerous reports describe the high heterogeneity and developmental and region-specific differences among microglia, astrocytes and OPC in the healthy and pathological CNS [106-108], which lead to different effects exerted by Gal-3 in these cells. Not only microglia seem to be involved in myelin phagocytosis; myelin uptake is an early response of astrocytes in diseases with prominent myelin injury, which results in the recruitment of immune cells [109]. Surprisingly, specific oligodendroglial lineage populations have been recently identified in the EAE model expressing genes involved in antigen processing and presentation (MHC-I and -II). It has also been demonstrated that OPC have phagocytic capacity and that MHC-II expressing OPC can activate memory and effector $CD4^+$ T cells [110]. Therefore, future experiments will be highly relevant in clarifying the possible involvement of Gal-3 in phagocytic functions in astrocytes and the oligodendroglial lineage.

The vast evidence available so far indicates that Gal-3 mediates the glial crosstalk and favors remyelination both by driving OLG differentiation and promoting a phagocytic microglial phenotype. These studies also shed light on some of the mechanisms underlying Gal-3 action and open doors for the identification of new signaling pathways regulated by Gal-3 to control OLG proliferation and differentiation (Figure 1). In this context, Gal-3 may constitute a therapeutic alternative, probably through the delivery of rGal-3 to OPC and microglia by nanocarriers and exosomes, as they can deliver cargo to other cells, easily cross the BBB and exhibit low immunogenicity. This is also supported by fact that Gal-3 can be excreted through exosomes. Altogether, these data unveil the therapeutic potential of Gal-3 in demyelinating diseases such as MS and may allow the development of new targets.

Author Contribution

LAP supported and wrote the manuscript.

Conflict of Interest

The author declares no conflict of interest.

Acknowledgements

The author wishes to thank Hernan Hoyos and Laura Thomas for dedicating their PhD studies to elucidating Gal-3 involvement in oligodendroglial biology. In addition, the author wants to thank Maria Marta Rancez for her help with article edition. This study was supported by grants from the Argentine Agency for the Promotion of Science and Technology (PICT 2016-0319) and the University of Buenos Aires (UBACYT- 20020170100285BA) to LAP.

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