

Perspective

Novel Galectin-3 interactions involved in oligodendroglial differentiation make inroads into therapeutic strategies for demyelinating diseases

Laura Andrea Pasquini*

Galectins (Gals) constitute a 15-member class of β -galactoside-binding lectins which recognize *N*-acetylglucosamine. Despite lacking specific receptors, Gals form multivalent complexes with cell surface glycoconjugates containing suitable oligosaccharides and thus trigger intracellular signals to regulate cell survival and differentiation. Gals are classified into three groups on the basis of their structural architecture: proto, chimera and tandem types, with Gal-3 being the only representative of the chimeric type. Regarding intracellular localization, Gal-3 is found in both cell cytoplasm and nucleus. Extracellular Gal-3 can also be endocytosed and, together with intracellular Gal-3, modulate diverse functions binding to intracellular molecules. While lacking a secretion signal peptide, Gal-3 is still secreted into the extracellular space by non-classical pathways potentially involving exosomes. Once secreted, Gal-3 binds to poly-*N*-acetylglucosamine in the extracellular matrix and membrane receptors, laying bridges to promote or inhibit intracellular events (Thomas and Pasquini, 2018).

Myelination is a physiological process by which lipid- and protein-rich myelin wraps axons and provides them with metabolic and trophic support. Having attained maturity through a succession of morphological and molecular changes, oligodendrocytes (OLG) are the cells in charge of central nervous system (CNS) myelination. Starting from highly proliferative platelet-derived growth factor receptor α (PDGFR α) and neural/glial antigen 2-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 myelin basic protein (MBP), adenomatous polyposis coli and myelin proteolipid protein expression and the ability to form myelin membranes (Franklin and Ffrench-Constant, 2017). A two-stage model has been proposed for actin dynamics in OLG: at an early stage, pro-polymerizing actin cytoskeleton dynamics foster OPC branching up to full OLG maturity; at a later stage, the actin cytoskeleton shifts to depolymerizing dynamics, which sparks myelination. These stages are partly regulated by the relative levels of MBP and actin disassembly proteins, e.g. cofilin-1 and gelsolin, commonly sequestered and inactivated by phosphatidylinositol 4,5-bisphosphate in the plasma membrane. MBP then competes for phosphatidylinositol 4,5-bisphosphate binding with gelsolin and cofilin-1 and displaces them to trigger the disassembly of actin filaments in m-OLG (Zuchero et al., 2018).

In 2011, our group pioneered the study of direct Gal-3 influence on OLG, showing that extracellular microglia-secreted Gal-3 promotes OLG differentiation, as wild type, but not LGALS3^{-/-} microglia-conditioned media, succeeded in inducing OLG maturation. In addition, Gal-3 expression appears to preserve myelin integrity and function, as evidenced by myelin ultrastructural and behavioral studies of LGALS3^{-/-} mice. Glycosylation signature analysis has shown that OPC possess a permissive glycophenotype expressing the necessary carbohydrates for Gal-3 binding (Pasquini et al., 2011). More recently, our group showed accelerated differentiation in recombinant Gal-3 (rGal-3)-treated OLG. In this study, rGal-3 administered to purified OPC once every 2 days accelerated differentiation,

as evidenced by an early increase in m-OLG markers and a decrease in immature OLG ones. These results coincided with accelerated F-actin dynamics, as revealed by an earlier polymerization peak and subsequent depolymerization, and were accompanied by an increase in the activation of Akt and the levels of β -catenin, MBP and gelsolin, as well as a decrease in the activation of Erk1/2 (Thomas and Pasquini, 2019). Even though how and when exactly the Akt/mTORC and Erk1/2 pathways participate in OLG differentiation and myelination remain controversial, a recent report has shown that PI3K/Akt, but not Mek/Erk1/2, plays a key role in promoting OLG differentiation. However, these two pathways work together converging at the level of mTORC1 to produce a mature and functional myelin (Thomas and Pasquini, 2020). In addition, other authors have postulated that Erk 1/2 inhibition promotes OLG generation and recovery in demyelinating processes (Thomas and Pasquini, 2020).

The timing and mechanisms underlying the pro-differentiating effects of Gal-3 were addressed through a series of treatment protocols using a single pulse of rGal-3 applied to different OLG maturation stages, i.e., OPC, pre-OLG and m-OLG. In these studies, rGal-3 was found to increase MBP expression, pAkt, β -catenin and F-actin, and to accelerate actin dynamics at both OPC and pre-OLG stages. Our results also indicate that mTORC1 is activated by rGal-3 treatment, which contributes to MBP expression and OLG maturation. However, single rGal-3 treatment at m-OLG stage failed to increase MBP expression, F-actin or β -catenin levels, and to activate Akt. Furthermore, all treatments induced an increase in gelsolin concomitantly with a decrease in pErk 1/2. These effects may be linked to increased MBP expression, as higher gelsolin levels positively correlate with OLG differentiation. Altogether, these results hint not only at a time window for rGal-3 action spanning OPC and pre-OLG stages, but also at interactions occurring throughout OLG maturation, which reinforces the key role of glycolipids and glycoproteins present at the time of treatment (Thomas and Pasquini, 2020).

Finally, direct rGal-3-molecule interactions were evaluated in OPC and pre-OLG by co-immunoprecipitation and subsequent mass spectrometry analysis. The main proteins found to interact with rGal-3 classified on the basis of the biological processes involved and their possible function in OPC are summarized in **Figure 1A** and **B** following the information previously described in Thomas and Pasquini (2020). Interestingly, the proteins identified at OPC stage are associated to cell proliferation, cytoskeleton, signaling cascades, energy metabolism and lipids and, probably, Gal-3 cellular internalization. At variance, proteins found to interact with rGal-3 at pre-OLG stage include those related to cytoskeleton, signaling cascades and rGal-3 cellular internalization, but none involved in cell proliferation. The characteristics of these proteins and their association to specific OLG stages support the notion that, upon internalization, extracellular rGal-3 is distributed into different subcellular compartments to interact with target molecules and thus mediate changes in the cytoskeleton, proliferation, lipid synthesis, and signaling pathways necessary to attain OLG

differentiation.

Oligodendroglial injury leads to demyelination, the pathological process by which myelin is lost from around axons (Franklin and Ffrench-Constant, 2017). Among demyelinating diseases, multiple sclerosis (MS) is one of the most common neurodegenerative CNS disorders, affecting 2.5 million people worldwide. Remyelination, the regenerative response consisting in the formation of new myelin sheaths, is essential for axonal survival and restoration of saltatory conduction, whereas its failure is a major cause of the neurological deficits in MS. 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.

The endogenous and exogenous roles of Gals in glial cells upon demyelination/remyelination have been recently documented in a thorough review by Jong et al. (2018). Serum from patients with secondary progressive MS has recently shown the presence of autoantibodies against Gal-3, probably responsible for blood-brain barrier progressive damage given their ability to bind to membrane Gal-3 in human brain microvascular endothelial cells (Thomas and Pasquini, 2018). Further evidence regarding the effect of cerebrospinal fluid from patients with primary progressive MS or relapsing remitting MS on OPC morphology and transcriptome has been provided. OPC treated with primary progressive MS cerebrospinal fluid exhibit more ramified morphology than those treated with control or relapsing remitting MS cerebrospinal fluid and show a pro-differentiating transcriptome, i.e. the downregulation of PDGFR α and LINGO1 genes and the upregulation of MAG gene, although this transcriptome differs from that found in normal OPC differentiation. Of note, this report shows LGALS3 gene upregulation only in OPC treated with primary progressive MS cerebrospinal fluid and postulates a connection between Gal-3 upregulation and OPC branching. Worth pointing out, Gal-3 upregulation has also been detected in post-mortem human brain tissues with primary progressive MS (Thomas and Pasquini, 2018). Likewise, when administered upon EAE immunization, FDA-approved drug for MS glatiramer acetate (copolymer 1) decreases Gal-3 expression in macrophages in the lesion site (Jong et al., 2018). Altogether, these findings hint at Gal-3 as a positive target for OLG differentiation in human MS tissue and point at the downregulation of ICAM-1 in brain microvascular endothelial cells as a means to protect the blood-brain barrier. However, the harmful effects of anti-Gal-3 auto-antibodies on the blood-brain barrier should be counteracted through neutralizing therapy.

Although MS experimental demyelination models fail to replicate the full complexity and heterogeneity of MS features, they have rendered interesting results and have allowed the development of various treatments. EAE applied on LGALS3^{-/-} mice rendered a reduction in CNS macrophage infiltration and disease severity, indicating a key role for Gal-3 in the promotion of inflammation by leukocyte recruitment (Thomas and Pasquini, 2018). At variance, further studies in this model revealed a neuroprotective role of Gal-3 through cell debris removal, axon regeneration and remyelination (Thomas and Pasquini, 2018). An increase in Gal-3 expression has been observed in active human MS lesions, periventricular regions in human MS and after virus-induced MS models like Theiler's murine encephalomyelitis virus, in which the loss of Gal-3 restores subventricular zone proliferation through a reduction in the number of immune cells (Thomas and Pasquini, 2018).

Our results have demonstrated that, during remyelination, microglia-expressed Gal-3 favors an M2 phenotype, hence fostering myelin debris phagocytosis through TREM-2b phagocytic receptor and OLG differentiation (Hoyos et al., 2014). Furthermore, recent work has identified Gal-3 as a novel endogenous TREM2 ligand (Boza-Serrano et al., 2019). Also recently, Gal-3 has been shown to activate phagocytosis by targeting the cytoskeleton

Perspective

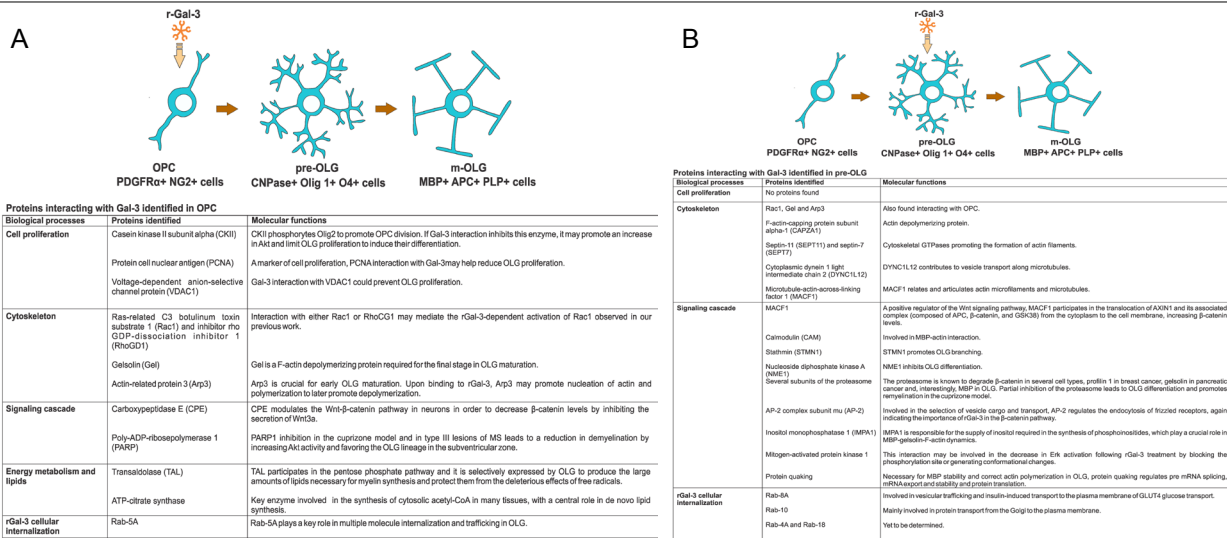


Figure 1 | Proteins interacting with rGal-3. (A) The main proteins found to interact with rGal-3, obtained through co-immunoprecipitation and mass spectrometry analysis of OPC lysates by Thomas and Pasquini (2019). These proteins are classified according to biological processes and possible molecular functions in OLG, following the information previously reported in Thomas and Pasquini (2019). (B) The main proteins found to interact with rGal-3, obtained through co-immunoprecipitation and mass spectrometry analysis of pre-OLG lysates by Thomas and Pasquini (2019). These proteins are classified according to biological processes and possible molecular functions in OLG, following the information previously described in Thomas and Pasquini, 2019. APC: Adenomatous polyposis coli; CNPase: 2',3'-cyclic-nucleotide 3'-Phosphodiesterase; MBP: myelin basic protein; m-OLG: mature OLG; mTOR: mammalian target of rapamycin complex; NG2: neural/glia antigen 2; OLG: oligodendrocyte; Olig: oligodendrocyte transcription factor; OPC: oligodendroglial progenitor; PDGFRα: platelet-derived growth factor receptor α; rGal-3: recombinant Galectin 3; TREM-2: triggering receptor expressed on myeloid cells-2.

twice: first, by advancing cofilin activation, enabling filopodia/lamellipodia to extend/engulf myelin debris; and second, by advancing actin/myosin-based contraction through K-Ras. GTP/PI3K signaling, causing filopodia/lamellipodia to retract/internalize myelin debris. Indeed, Gal-3 knock-down leads to a sharp shift in microglial morphology from amoeboid to branched-like, the rearrangement of actin filaments and the inactivation of cofilin, which then fails to trigger phagocytosis (Reichert and Rotshenker, 2019).

Recent work has demonstrated that overexpressing Gal-3 with electroporation in subventricular zone showed no inflammation in the healthy postnatal increased gliogenesis with an increased percentage of striatal astrocytes but a decreased percentage of OLG, mediated by an increased bone morphogenetic protein signaling (Al-Dalahmah et al., 2019).

Given that Gal-3 binds to multiple targets, its contrasting effects on OLG biology and myelination may reflect direct and indirect actions, i.e. those mediated by microglia, astrocytes or peripheral cells. Furthermore, the high heterogeneity and developmental and region-specific differences among microglia, astrocytes and OPC may have consequences in Gal-3 effects. Worth highlighting, our results show that an increase in intracellular Gal-3 levels does not produce changes in the expression of m-OLG marker MBP, and that only extracellular Gal-3 treatment accelerates OLG differentiation (Pasquini et al., 2011). In addition, this pro-differentiating effect is observed within a certain range of rGal-3 concentrations, above which the viability of the OLG is impaired, and within a specific time window along the oligodendroglial lineage (Pasquini et al., 2011; Thomas and Pasquini, 2019, 2020). Moreover, the biological activity of Gal-3 depends on metalloprotease cleavage and the interaction with mediators of the extracellular matrix (Pasquini et al., 2011; Thomas and Pasquini, 2018). Therefore, studies into the therapeutic potential of Gal-3 in demyelinating diseases should consider the delivery of rGal-3 to OPC, for example, through nanocarriers and exosomes able to cross the blood-brain barrier and targeted with specific antibodies such as anti-neural/glia antigen 2 or anti-PDGFRα. Exosomes have recently emerged as possible biomarkers and therapeutic agents in CNS disease. Moreover, exosomes pose therapeutic advantages, as they can deliver cargo to other cells, easily cross the blood-brain barrier and exhibit low immunogenicity (Thomas and Pasquini, 2018). The

fact that Gal-3 can be excreted through exosomes thus paves the way for studies into the therapeutic potential and biomarker capacity of exosomal Gal-3. Alternatively, microglia should also be considered as a potential target, as Gal-3 induces an increase in their phagocytic capacity and fosters the clearance of myelin debris produced by demyelination, a key step in the onset of remyelination.

Altogether, findings so far indicate that Gal-3 mediates the glial crosstalk and favors remyelination both by driving OLG differentiation and promoting a phagocytic microglial phenotype which facilitates the onset of remyelination. These results 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. This knowledge may allow the development of new targets in the design of future therapies for demyelinating diseases such as MS.

This work was supported by grants from Agencia Nacional de Promoción Científica y Tecnológica (Argentina, PICT 2016-0319) and Universidad de Buenos Aires (20920160100683BA, to LAP).

Laura Andrea Pasquini*

Department of Biological Chemistry, School of Pharmacy and Biochemistry, Institute of Biological Chemistry and Physicochemistry (IQUIFIB), University of Buenos Aires and National Research Council (CONICET), Buenos Aires, Argentina

*Correspondence to: Laura Andrea Pasquini, PhD, laupasq@yahoo.com.

<https://orcid.org/0000-0003-4292-3463> (Laura Andrea Pasquini)

Received: February 5, 2020

Peer review started: February 24, 2020

Accepted: April 13, 2020

Published online: August 24, 2020

<https://doi.org/10.4103/1673-5374.290887>

How to cite this article: Pasquini LA (2021) *Novel Galectin-3 interactions involved in oligodendroglial differentiation make inroads into therapeutic strategies for demyelinating diseases.* *Neural Regen Res* 16(2):289-290.

Copyright license agreement: The Copyright License Agreement has been signed by the author before publication.

Plagiarism check: Checked twice by iThenticate.

Peer review: Externally peer reviewed.

Open access statement: This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

Open peer reviewers: Wensheng Lin, University of Minnesota, USA; Tianci Chu, University of Louisville School of Medicine, USA; Yi Pang, The University of Akron, USA.

Additional file: Open peer review reports 1–3.

References

Al-Dalahmah O, Campos Soares L, Nicholson J, Draijer S, Mundim M, Lu VM, Sun B, Tyler T, Adorján I, O'Neill E, Szele FG (2020) Galectin-3 modulates postnatal subventricular zone gliogenesis. *Glia* 68:435-450.

Boza-Serrano A, Ruiz R, Sanchez-Varo R, García-Revilla J, Yang Y, Jimenez-Ferrer I, Paulus A, Wennström M, Vilalta A, Allendorf D, Davila JC, Stegmayr J, Jiménez S, Roca-Ceballos MA, Navarro-Garrido V, Swanberg M, Hsieh CL, Real LM, Englund E, Linse S, et al. (2019) Galectin-3, a novel endogenous TREM2 ligand, detrimentally regulates inflammatory response in Alzheimer's disease. *Acta Neuropathol* 138:251-273.

de Jong CGHM, Gabius HJ, Baron W (2019) The emerging role of galectins in (re)myelination and its potential for developing new approaches to treat multiple sclerosis. *Cell Mol Life Sci doi: 10.1007/s00018-019-03327-7*.

Franklin RJM, Ffrench-Constant C (2017) Regenerating CNS myelin from mechanisms to experimental medicines. *Nat Rev Neurosci* 18:753-769.

Hoyos HC, Rinaldi M, Mendez-Huergo SP, Marder M, Rabinovich GA, Pasquini JM, Pasquini LA (2014) Galectin-3 controls the response of microglial cells to limit cuprizone-induced demyelination. *Neurobiol Dis* 62:441-455.

Pasquini LA, Millet V, Hoyos HC, Giannoni JP, Croci DO, Marder M, Liu FT, Rabinovich GA, Pasquini JM (2011) Galectin-3 drives oligodendrocyte differentiation to control myelin integrity and function. *Cell Death Differ* 18:1746-1756.

Reichert F, Rotshenker S (2019) Galectin-3 (MAC-2) Controls microglia phenotype whether amoeboid and phagocytic or branched and non-phagocytic by regulating the cytoskeleton. *Front Cell Neurosci* Mar 13:90.

Thomas L, Pasquini LA (2018) Galectin-3-mediated glial crosstalk drives oligodendrocyte differentiation and (re)myelination. *Front Cell Neurosci* 12:297.

Thomas L, Pasquini LA (2019) Extracellular Galectin-3 induces accelerated oligodendroglial differentiation through changes in signaling pathways and cytoskeleton dynamics. *Mol Neurobiol* 56:336-349.

Thomas L, Pasquini LA (2020) Galectin-3 exerts a pro-differentiating and pro-myelinating effect within a temporal window spanning precursors and pre-oligodendrocytes: insights into the mechanisms of action. *Mol Neurobiol* 57:976-987.

Zuchero JB, Fu MM, Sloan SA, Ibrahim A, Olson A, Zaremba A, Dugas JC, Wienbar S, Capriarello AV, Kantor C, Leonoudakis D, Lariosa-Willingham K, Kronenberg G, Gertz K, Soderling SH, Miller RH, Barres BA (2015) CNS myelin wrapping is driven by actin disassembly. *Dev Cell* 34:152-167.

P-Reviewers: Lin W, Chu T, Pang Y; C-Editors: Zhao M, Li JY; T-Editor: Jia Y