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Research Paper

Ligand-mediated Galectin-1 endocytosis prevents intraneural H_2O_2 production promoting F-actin dynamics reactivation and axonal re-growth

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ABSTRACT

Axonal growth cone collapse following spinal cord injury (SCI) is promoted by semaphorin3A (Sema3A) signaling via PlexinA4 surface receptor. This interaction triggers intracellular signaling events leading to increased hydrogen peroxide levels which in turn promote filamentous actin (F-actin) destabilization and subsequent inhibition of axonal re-growth. In the current study, we demonstrated that treatment with galectin-1 (Gal-1), in its dimeric form, promotes a decrease in hydrogen peroxide (H_2O_2) levels and F-actin repolimerization in the growth cone and in the filopodium of neuron surfaces. This effect was dependent on the carbohydrate recognition activity of Gal-1, as it was prevented using a Gal-1 mutant lacking carbohydrate-binding activity. Furthermore, Gal-1 promoted its own active ligand-mediated endocytosis together with the PlexinA4 receptor, through mechanisms involving complex branched N-glycans. In summary, our results suggest that Gal-1, mainly in its dimeric form, promotes re-activation of actin cytoskeleton dynamics via internalization of the PlexinA4/Gal-1 complex. This mechanism could explain, at least in part, critical events in axonal regeneration including the full axonal re-growth process, *de novo* formation of synapse clustering, axonal re-myelination and functional recovery of coordinated locomotor activities in an *in vivo* acute and chronic SCI model.
Significance statement: Axonal regeneration is a response of injured nerve cells critical for nerve repair in human spinal cord injury. Understanding the molecular mechanisms controlling nerve repair by Galectin-1, may be critical for therapeutic intervention. Our results show that Galectin-1; in its dimeric form, interferes with hydrogen peroxide production triggered by Semaphorin3A. The high levels of this reactive oxygen species (ROS) seem to be the main factor preventing axonal regeneration due to promotion of actin depolymerization at the axonal growth cone. Thus, Galectin-1 administration emerges as a novel therapeutic modality for promoting nerve repair and preventing axonal loss.

1. Introduction

Although underappreciated for many years, emerging observations suggest essential roles for glycan-binding proteins and their corresponding glycosylated ligands in different central nervous system (CNS) pathologies (Rabinovich and Croci, 2012; Mendez-Huergo et al., 2014). In previous studies, we reported the functional recovery of mice with spinal cord injury (SCI) following local treatment with galectin-1 (Gal-1), a highly conserved glycan-binding protein, through mechanisms involving interruption of Semaphorin3A (Sema3A)-driven inhibitory signals (Quinta et al., 2014b). We found that Gal-1 binds to the Neuropilin-1 (NRP-1)/PlexinA4 receptor complex on the surface of injured neurons through a glycan-dependent mechanism, promoting axonal regeneration. However, the molecular mechanism through which Gal-1 association with NRP-1/PlexinA4 promotes axonal re-growth remains uncertain.

It is well known that Sema3A, a chemo-repulsive axonal guidance molecule, induces growth cone collapse after SCI (Takahashi et al., 1999). This protein is secreted by meningeal fibroblasts spreading out at the lesion site (Pasterkamp et al., 1999), where it binds to NRP-1/PlexinA4 surface receptor in the injured axonal tracts (Tamagnone et al., 1999; Pasterkamp and Verhaagen, 2001; De Winter et al., 2002a; De Winter et al., 2002b). This interaction, leads to intracellular signaling events that raise MICAL activity,

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thus promoting an increase in intra-axonal hydrogen peroxide (H_2O_2) production, which in turn oxidizes F-actin at methionine residues, resulting in F-actin destabilization. The whole process generates growth cone collapse (sharp decrease in F-actin length and bundling) and inhibition of axonal re-growth (Hung and Terman, 2011; Giridharan and Caplan, 2014). Moreover, neuronal H_2O_2 production over physiological levels is closely associated with axonal growth failure (Hung et al., 2010; Hung et al., 2011; Morinaka et al., 2011), supporting a link between inhibition of axonal regeneration and production of reactive oxygen species (ROS).

From a biochemical viewpoint, Gal-1 is an endogenous homodimeric lectin composed of subunits of 14.5 kDa that binds to glycosylated receptors displaying multiple units of the common disaccharide (Galβ1–4)GlcNAc; LacNac) on both *N*- and *O*-glycans (Rabinovich and Croci, 2012). Within the CNS, injection of Gal-1 following SCI, binds to the NRP-1/PlexinA4 complex, promoting axonal regeneration and recovery of locomotor activities (Quinta et al., 2014b). However, in spite of considerable progress the molecular mechanisms underlying this neuro-regenerative effect remain uncertain.

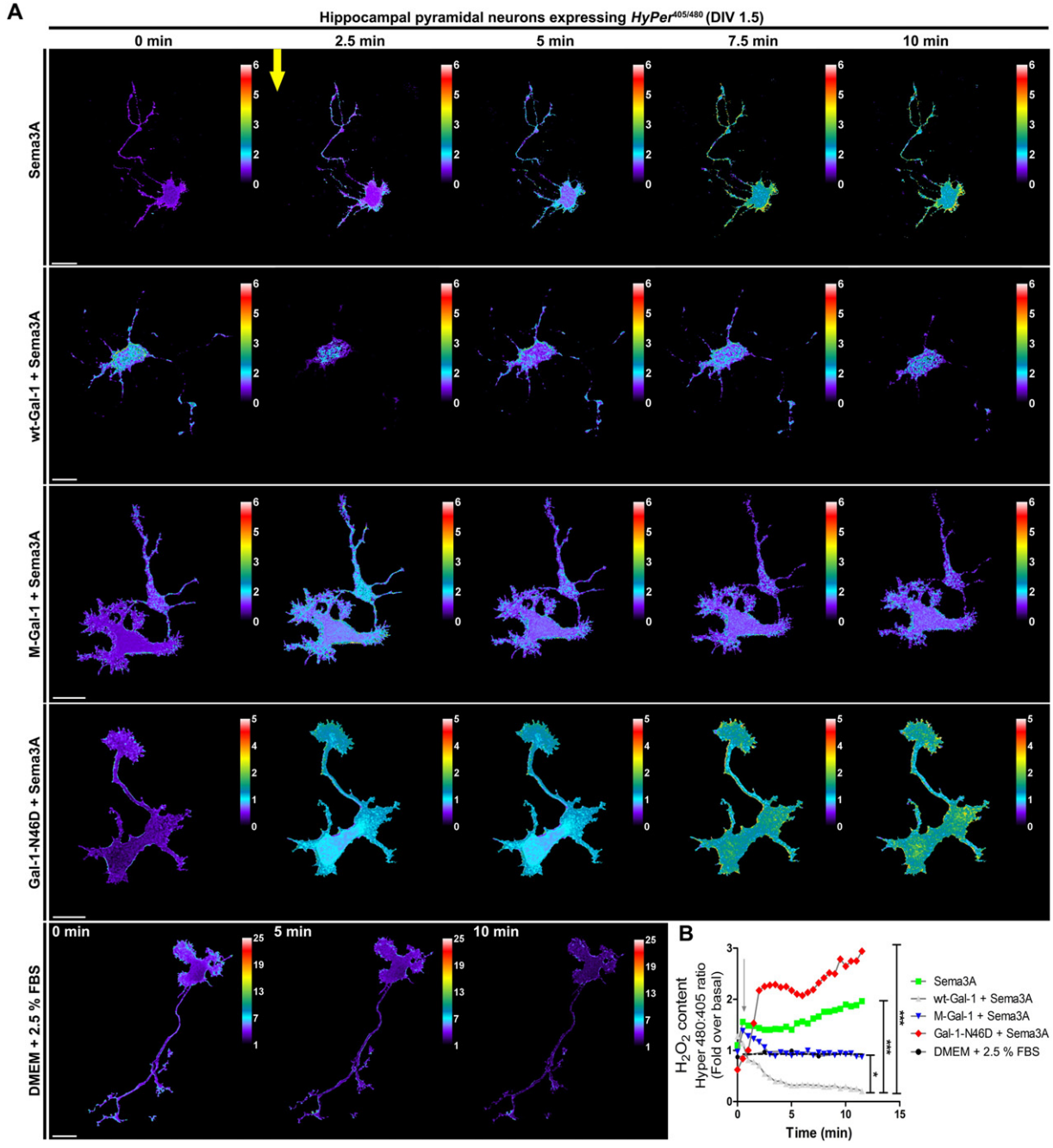

Fig. 1. Real time measurement of H_2O_2 production in hippocampal pyramidal neurons. (A) H_2O_2 content evaluated by confocal time-lapse acquisition of representative HyPer transfected neurons. The experimental conditions were: 300 μg/ml of Sema3A (first line); 280 μg/ml of wt-Gal-1 (15 min of pre-incubation) + 300 μg/ml of Sema3A (second line); 280 μg/ml of M-Gal-1 (15 min of pre-incubation) + 300 μg/ml of Sema3A (third line); 280 μg/ml of Gal-1-N46D (15 min of pre-incubation) + 300 μg/ml of Sema3A (fourth line). The yellow line indicates Sema3A addition. Basal H_2O_2 content control evaluated in HyPer transfected neurons (fifth line). In the right corner of each picture, a calibration bar is shown (0–255 colors) normalized for each condition. Scale bar, 20 μm. (B) Graphical analysis shows a quantification of H_2O_2 content 480:405 ratio (fold over basal). The gray arrow in the bar graph indicates Sema3A addition. Values represent the mean of three independent experiments (n = 5 transfected cells analyzed per condition). $^{***}P < 0.05$, $^{*}P < 0.05$ using one-way ANOVA followed by Dunn's multiple comparison tests.