PERSPECTIVE

Past, present and future of preserving and restoring function in the visual system: removing galectin-3 as a promising treatment

Great advances in retinal ganglion cells survival (RGCs), optic nerve preservation and regeneration have been made in the past 15 years. Nowadays, we know that RGCs are capable of regenerating the full length of the optic nerve, cross the chiasm, enter the brain and reinnervate visual targets. In order to obtain successful regeneration, RGCs need to activate signaling pathways related to cell survival and turn on their intrinsic growth capacity. Studies that aimed at blocking cell death and inhibiting apoptosis by B-cell lymphoma 2 (Bcl-2) overexpression showed an increase in cell survival, but these approaches were not sufficient to promote axon regeneration, even when axons were put in an permissive environment, as peripheral nerve graft (Inoue et al., 2002). Neither stimulating axon regeneration by intraocular inflammation, nor delaying axon degeneration by overexpression of *Wlds* protein, or inhibition of calpain activation (**Figure 1C**) (de Lima et al., 2016) showed any increase in cell survival. Nevertheless, Park et al. (2008) showed that phosphatase and tensin homolog (PTEN) gene deletion on RGCs stimulates both cell survival and axon regeneration (Benowitz et al., 2016 for review).

The scenario revealed by these studies indicates that different mechanisms regulate RGC survival and axon regeneration. From these evidences, investigators started to combine different treatments, focusing on cell survival, axon regeneration, or both. The rationale behind this approach is that one would be able to stimulate both RGCs survival and axon regeneration at the same time, and possibly get additional effect after a lesion to the optic nerve. For instance, specific single treatments, such as conditional deletion of the PTEN gene in RGCs resulted in 45% of cell survival after optic nerve crush (ONC), and also promoted modest axon regeneration (Park et al., 2008). However, when combined with intraocular inflammation, a RGC survival rate of 54% was achieved as well as a 10 fold increase in axon regeneration, resulting in brain reinnervation (de Lima et al., 2012) (**Figure 1E**). Therefore, a combination of treatments can be a powerful tool to stimulate recovery of visual pathway. Thus, researchers are focusing their efforts on identifying potential candidates that can be more effective in one aspect (cell survival), in the other (axon regeneration), or both, so they can combine those candidates and boost brain target reinnervation.

Successful regeneration of RGCs and brain reinnervation: There are few treatments that successfully stimulated the regeneration of the full length of the optic nerve, reaching subcortical visual targets (de Lima et al., 2012; Li et al., 2015; Bei et al., 2016; Lim et al., 2016). Although different groups have shown some level of brain reinnervation, there are still a lot of controversies if regenerated cells are able to find their targets and recover function (de Lima et al., 2012; Bei et al., 2016). While de Lima et al. (2012) showed evidence that RGCs can extend axons all the way from the eye to subcortical visual targets and become remyelinated, some studies, using different treatments, claimed that the axons cannot be remyelinated (Bei et al, 2016), get stuck at the chiasm and are unable to find their way to visual targets (Luo et al., 2013). However, other studies have reported a complete regeneration of RGCs, visual targets reinnervation and partial recovery of visual behaviors, activating mechanistic target of rapamycin (mTOR) signaling pathway combined with enhancement of neural activity (Lim et al, 2016). More studies have to be done in order to understand, not only if specific treatments can induce remyelination but, more widely why RGCs behave differently when subjected to a specific treatment – for instance: 1) why total number of surviving RGCs and specific RGCs subtypes can vary among studies? 2) Why only some treatments can promote long distance regeneration? 3) How regenerating axons interact with myelinating oligodendrocytes in order to become myelinated? 4) How regenerating axons are guided towards visual targets? 5) Does stimulation of activity improves regeneration? 6) How much axon regeneration is enough to produce significant functional recovery? 7) Does specific subtypes of RGCs change their profile to compensate for specific subtypes that were lost? Answering these questions might help us to achieve the recovery of the visual function after a lesion to the optic nerve, or in the case of the neurodegenerative diseases, such as diabetic retinopathy, which are the leading causes of blindness in adults. Nevertheless, these studies have shown that the rate of cell survival decreases overtime, with only 34% of cells surviving at 10–12 weeks after injury (de Lima et al., 2012). So, different approaches are still required to achieve a satisfactory visual function recovery after lesions to the optic nerve.

Galectin-3 (Gal-3) deletion and visual system preservation: Previous work from our group showed that Gal-3 absence increased peripheral nerve regeneration after crush (Narciso et al., 2009) and improved white matter sparing and motor function after spinal cord lesion (Mostacada et al., 2015). These results, in addition to the knowledge that Gal-3 is required for microglia/macrophage phagocytic activity during optic nerve Wallerian degeneration (WD), led us to hypothesize that the absence of Gal-3 could reduce RGCs death and modulate axonal degeneration/regeneration after ONC. This subject was investigated in a study in Abreu et al. (2016), where we showed that the absence of Gal-3 promotes neuroprotection of RGC, since Gal-3$^{-/-}$ mice presented an increase in the survival of RGC after injury and less apoptotic RGC in their retinas. Importantly, the authors found the highest survival level of RGCs ever promoted by a single treatment after optic nerve crush - that is 52% two weeks after lesion. Albeit surviving, no GAP-43 positive axons were observed in neither group, indicating that Gal-3$^{-/-}$ RGC axons were not able to regenerate (**Figure 1D**). The course of WD within the optic nerve was also analyzed, and the quantitative analysis of the ultrastructure of Gal-3 knockout optic nerves two weeks after crush showed an increased number of myelinated identifiable fibers, although presenting degenerating axoplasm, indicating that the WD process is impaired in Gal-3 knockout mice 2 weeks after optic nerve injury. The neuroprotection reported by our study might be related to a decrease in the inflammatory response caused by lower number of inflammatory cells, such as microglia and macrophage at the site of the lesion, associated with less astrogliosis, which resulted in slow degeneration of optic nerve fibers and RGC survival. It is of great interest to understand the mechanisms by which the increase in cell survival is taking place. It can be inferred that the effect on cell survival is most likely a tissue extrinsic mechanism, since Gal-3 is not expressed in both retinal ganglion cells or in the optic nerve (unpublished observations). Since there was an important effect on cell survival, it would be interesting to know for how long it can be maintained, in order to have Gal-3 knockdown in association with other therapeutic approaches. If this effect on RGCs survival is sustained overtime, it would be of great interest to associate Gal-3 knockdown, knockout or blockade with one or more pro-regenerative therapies that have proved to be effective, such as intraocular inflammation, PTEN or SOCS3 conditional deletion within RGCs, mTOR overactivation or enhancement of visual activity.

Besides, our results raised the possibility to study the role of Gal-3 in other important visual incapacitating diseases, such as diabetic retinopathy. Estimated to affect 422 million people around the globe, diabetes is one of the main pandemics of the XXI century. Considered the most common cause of acquired blindness, diabetic retinopathy is responsible for 1.9% of the global cases of severe

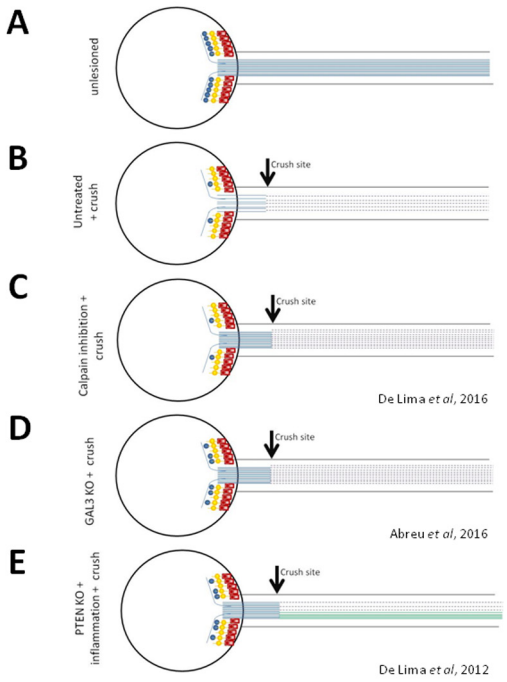

Figure 1 Profile of RGC death and regeneration after crush under different conditions.
(A) Normal retinofugal pathway. Note the presence of retinal ganglion cell (RGC) within the retina and their axons projecting along the optic nerve. (B) After optic nerve crush, few RGCs survive and no axons regenerate beyond the crush site, where only degenerated axons are present. (C) Calpain inhibition prevents fiber degeneration, but has no effect on RGC survival after crush. (D) Galectin-3 (Gal3) knockout prevents both fiber degeneration and RGCs death, but do not stimulate axon regeneration beyond the lesion site. (E) Phosphatase and tensin homolog (PTEN) conditional deletion on RGCs alone or combined with intraocular inflammation promotes both RGCs survival and axon regeneration. Red cells represents photoreceptors, yellow cells represents bipolar cells, blue cells represent RGCs, blue lines represent RGCs axons inside the eye and on the optic nerve, dotted lines represent degenerating fibers, while green lines represent regenerating fibers. Amacrine, horizontal and glial cells are not represented.

visual impairment worldwide and 2.6% of total blindness cases. Recently, high serum levels of Gal-3 has been tagged as a new risk factor associated with pre-diabetes and diabetes in human subjects (Yilmaz et al., 2014). Indeed, the infiltration of inflammatory cell and β pancreatic cell death are reduced in Gal-3 knockout mice, decreasing the susceptibility to diabetes induction (Mensah-Brown et al., 2009). Moreover, the number of microglia/macrophage are drastically reduced in the lesion site of both spinal cord (Mostacada et al., 2015) and optic nerve (Abreu et al., 2016) of the Gal-3 knockout mice. Also, absence of Gal-3 has been shown to polarize both macrophage and microglia to an M2 phenotype after both spinal cord injury and diabetes induction in mice (Mensah-Brown et al., 2009; Mostacada et al., 2015). Since previous research from our group has shown preservation of CNS white matter in the spinal cord (Mostacada et al., 2015) and optic nerve (Abreu et al., 2016), also rescuing RGCs from apoptosis after lesion, it would be of interest to investigate if Gal-3 absence could relieve the ophthalmological symptoms of diabetes, focusing studies in visual function, neurodegeneration, neuroregeneration, neuroplasticity and neuroinflammation.

Since Gal-3 inhibitors, such as GR-MD-02, are available, even being used in clinical trials (Harrison et al., 2016), the identification of Gal-3 as a potential target to treat neurodegenerative diseases, may open new possibilities for translational studies and clinical treatments.

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