Glial regenerative response in the imaginal discs of Drosophila melanogaster

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Glial cells play a key role during nervous system development and actively participate in all the cellular processes involved in maintaining structural robustness and functional plasticity. In response to neuronal damage, glial cells proliferate, migrate to the injured region and change their morphology, function, and behavior (Gallo and Deneen, 2014; Kato et al., 2018). This glial regenerative response is associated with the reparation function of these cells and is found across species, suggesting that it may reflect a common underlying genetic mechanism (Kato et al., 2018). In mammals, while the central nervous system has very limited capacity to regenerate after traumatic injury or disease, the peripheral nervous system (PNS) exhibits a far greater capacity for regeneration and damaged peripheral nerves can be totally restored (Brosius Lutz and Barres, 2014; Gallo and Deneen, 2014). The PNS largely owes its regenerative potential to the ability of the main glial cells present in the PNS, myelin, and non-myelin (Remak) Schwann cells, to convert to cells devoted to repairing after injury (Nocera and Jacob, 2020). During the regeneration of peripheral nerves in vertebrates, Schwann cells function as a central hub, collecting signals from neurons and other cell types and undergoing a complex process of reprogramming which converts them into a specialized cell for repair. Even though many aspects of regeneration in peripheral nerves have been studied, there is still a lack of understanding regarding the genetic network that controls the flexible differentiation state of PNS neurons and Schwann. The identification of those signals is essential for getting new insight to develop innovative regenerative therapies. In this scenario, the use of relatively simple model organisms, amenable to genetic, cellular, and molecular analysis is fundamental to study the behavior of glial cells in response to damage in their natural context.

The fruit-fly Drosophila offers a powerful system to investigate in vivo fundamental biology, and has proven to be an extremely powerful model organism to discover evolutionary conserved gene function and networks. Consequently, this organism has been used to characterize elementary features of cell biology, including basic mechanisms that regulate damage response in the central nervous system (Kato et al., 2018). Additionally, the regenerative response after damaging the PNS has also been analyzed in Drosophila. Most of these studies have focused on the resulting degeneration and regeneration of axons and dendrites observed after damage (Fang and Bonini, 2012; Brace and DiAntonio, 2017). However, the response of glial cells upon PNS damage in terms of proliferation, migration, and functional changes, as well as the molecular and genetic mechanisms that might regulate this response have remained largely unexplored up to date. Thereupon, we approached this issue by exploring the behavior of glial cells after inducing neural apoptosis in different regions of the PNS. With this aim, we first used the eye imaginal discs as a biological model.

The adult eye of Drosophila develops from the imaginal eye discs (Figure 1). The cells that form the eye discs proliferate throughout most of larval life as a simple epithelial sheet and only begin to differentiate in the third instar. During this stage, a population known as the morphogenetic furrow (MF) sweeps across the eye field from posterior to anterior triggering the onset of neural differentiation. Thus, posterior to the MF cells begin to differentiate as neurons (photoreceptors) and accessory cells that will form the individual units of the compound eye, known as ommatidia. In addition to these cell types, the eye discs also contain glial cells (Figure 1). Unlike the photoreceptors and the other cells that will form the retina, the precursors of all subretinal glial cells are not generated from the eye disc cells, since they are specified during embryogenesis in the nerve that connects the primordium of the eye disc to the brain (Bolwig nerve). This nerve will become the optic stalk and will maintain the developing imaginal disc connected to the brain (Silies et al., 2010). The precursor glial cells proliferate in the optic stalk during larval life as a simple epithelial sheet and only begin to differentiate in the third instar. During this stage, the cells that form the morphogenetic furrow (MF) will become the optic stalk and will maintain the developing imaginal disc connected to the brain (Silies et al., 2010). The precursor glial cells proliferate in the optic stalk during larval life as a simple epithelial sheet and only begin to differentiate in the third instar. During this stage, the cells that form the morphogenetic furrow (MF) will become the optic stalk and will maintain the developing imaginal disc connected to the brain (Silies et al., 2010).

There are three main types of glial cells in the eye disc: perineurial glia, perineurial glia, and wrapping glia (WG). Perineurial cells, the so-called carpet cells, are two large cells that cover the entire differentiated part of the eye disc epithelium. Below these cells, we find the perineurial glia, which maintains the ability to divide during eye disc development. These cells will eventually form the wrapping glia cells. These latter glial cells enwrap all axons produced by the photoreceptors (Yildirim et al., 2018). Interestingly, wrapping glial cells perform functions, which resemble the non-myelinating Schwann cells forming Remak fibers in the mammalian PNS (Yildirim et al., 2018). Morphologically, it has been described as two more glial cell types. The function of these cells is unknown (Silies et al., 2010; Yildirim et al., 2018).
The development of the glial cells in the eye discs has been used as a model system to study the mechanisms that regulate the coordinated development of neurons and glial cells (Silies et al., 2010; Bauke et al., 2015). However, the response of glial cells to neuronal damage in the retina and the signaling pathways that might be mediating this function has remained unexplored.

Alternative to the eye disc, the behavior of PNS glial cells to neural damage appears to be studied in the developing Drosophila leg imaginal disc. These epithelial structures contain different sense organs arranged in a precise and defined fashion. Each sense organ is formed by neuron and glial cells that, like eye discs, are specified during larval development in the nerve that connects the leg disc anlage and the sensory organs. As in eye disc, glial cells have to migrate through the nerve into the forming leg (Sasse and Klambt, 2016).

We took advantage of the rich palette of genetic tools available in Drosophila to induce targeted cell death of photoreceptors and retinal cells of the eye disc, as well as of epithelial cells of the leg discs, including neurons of the sensory organs. To this end, we used the Ga4/UAS/Gal80ts system to conditionally delete the pro-apoptotic gene reaper (rpr) under the control of Ga4 lines that were specifically expressed in the eye or leg discs (Velarde et al., 2021). Since glial cells do not contribute to the embryonic pro-apoptic signaling, these drivers are not active in glial cells, and therefore any change in glial behavior is due to the signals emitted by dying neural tissue. In this sense, the eye disc is an excellent model to define the signals produced by damaged neuronal tissue that promote the response of the glial cells.

We found that cell death induction, in both the retinal region of eye discs and in leg discs, results in the accumulation of glial cells in the eye and leg discs. This effect is converse to an increased glial proliferation and over migration. However, in leg discs we have not detected an excess proliferation in the leg discs epithelium, but not in the discs, these drivers are not active in glial cells, and therefore any change in glial behavior is due to the signals emitted by dying neural tissue. In this sense, the eye disc is an excellent model to define the signals produced by damaged neuronal tissue that promote the response of the glial cells.

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In line with previous observations in other regions of the PNS (Macdonald et al., 2013), we found that in response to cell death in the retina or in leg disc, c-Jun N-terminal kinase (JNK) signaling is activated in the damaged region, as well as in glial cells. We assayed the activity of this signaling using reporters that contain binding sites for Activator Protein 1 (a heterodimeric Jun/Fos transcription factor targeted by JNK). Our data suggest that in response to damage, JNK signaling can activate a transcriptional program that promotes a specific aspect of the glial response. We found that JNK signaling down-regulation in either the eye discs or in glial cells reduces the accumulation of glial cells observed upon neural damage, without affecting glial proliferation. This observation suggests that in response to neural damage, JNK signaling is necessary to promote glial motility but not proliferation. This effect seems to be only permissive, as the ectopic activation of JNK signaling was not sufficient for increasing glial motility.

Our observations indicate that upon apoptosis induction, a diffusible signal is generated that not only activates JNK signaling in the damaged region, but also in glial cells. Surprisingly, we found that the activation of this pathway in Drosophila, the tumor necrosis factor termed Eiger, is not the signal emitted from the damaged region, as the reduction of its activity does not affect glial response. We have not established what the mechanism by which the transduction pathway of this signal is activated in the damaged tissue or in glial cells. Interestingly, the ectopic expression of dpp in the retina and dpp in the PNS, that in response to damage induces the migration of glial cells upon dpp over-expression, as the over-migration phenotype caused by the ectopic activation of dpp signaling in glial cells is totally suppressed when JNK signaling is blocked.

Neuronal apoptosis also causes changes in the glial architecture and proliferation of glial cells. We found that most wrapping and some perineurial glial cells expand their membrane surface, generating glial processes (Figure 1). Moreover, they develop phagocytic abILITIES and can engulf and phagocytize apoptotic debris. These behavioral and morphological changes resemble the regenerative response of non-myelinating Schwann cells in vertebrate PNS (Nocera and Jacob, 2020). The ability of Schwann glia to convert into cells specialized to support regeneration is a fundamental cell fate in vertebrates to activate JNK signaling in glial cells, although the details of the mechanism remain unclear. This regenerative mechanism might promote the migration of glial cells upon dpp over-expression, as the over-migration phenotype caused by the ectopic activation of dpp signaling in glial cells is totally suppressed when JNK signaling is blocked.

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