Regenerative capacity of Müller cells and their modulation as a tool to treat retinal degenerations

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Vision is one of our most precious senses, and its impairment has a high socio-economic impact. In the industrialized world, degenerative diseases of the eye impose a significant burden, particularly affecting the elderly. These degenerations include, for instance, retinitis pigmentosa, age-related macular degeneration, and diabetic retinopathy. Although treatments are evolving to manage late-stage symptoms of retinal degenerations, no effective therapies to recover vision loss exist. Retinal degeneration often involves loss or damage to specialized neural cells, such as photoreceptors, and their death stimulates the activation and proliferation of Müller cells (Salman et al., 2021).

Müller cells are the predominant glial cell type in the retina, constituting about 30% of the retinal glia. They are radially oriented and span the entire depth of the neural retina. Initially, Müller cells were believed nothing more than an adhesive scaffold for retinal cells. However, numerous studies show that Müller cells have more functions, including homeostatic regulation, metabolic support to the retina, and light transmission under photoreceptor conditions. They can also alter the properties of a retinal stem cell. Furthermore, Müller cells are involved in response to injury, carrying out either a protective or detrimental function (Liu et al., 2021). Importantly, in zebrafish, they adopt certain progenitor/stem cell features, dislocate to the damaged retinal area, and promote the transcription of genes and proteins involved in axon guidance and patterning of mammalian Müller cells demonstrated evidence for their neurogenic potential. However, it is still unclear why the regenerative response in mammals is minimal compared to the robust response in fish (Hoang et al., 2020). In mammals, Müller cells are also the glial cell type primarily involved in gliosis. Gliosis is a pathophysiological feature of glial cells and is the hallmark of neuronal loss and consequent proliferation of Müller cells in response to injury and/or disease. Müller cell reactivity differs between species and even among individual cells according to different pathological stimuli. The activated glial cells give a multifactorial signal, which potentially includes inflammatory factors and development of glial scar. This contributes to further degeneration and impedes tissue regeneration (Graca et al., 2018). Upon injury, Müller cells also generate neurotrophic factors to promote recovery. The complex molecular machinery that regulates retinal regeneration in fish and glial scar formation in mammals is undetermined to date, stimulating more research into the topic. Diverse molecular pathways drew the attention of researchers, such as tumor necrosis factor-alpha (TNFα), Wnt, and JAK-STAT pathways. In this perspective, we are going to focus mainly on the transforming growth factor-beta (TGFβ)-Smad3-Notch axis.

TGFα belongs to a group of pleiotropic cytokines and regulates several cellular processes during embryogenesis and adulthood. TGFβ signaling is necessary for wound healing, including non-specific scar formation and tissue-specific regeneration. The TGFβ superfamily comprises 33 members: three multi-functional isoforms TGFβ1, TGFβ2, and TGFβ3, three activins/ inhibins, and several non-collagenous and non-collagenal signaling. This pathway affects the immune response, scar formation, and muscle development (Gilliland & Beachy, 2016). The specific TGFβ isoforms and downstream mediators play different roles in each of these processes. For instance, in mammals, TGFβ1 and TGFβ2 promote collagen deposition and scar formation, while TGFβ3 is anti-fibrotic (Yang et al., 2020). In zebrafish, the TGFβ pathway is involved during heart, fin, and retina regeneration. In a light-induced model of retinal injury in zebrafish, TGFβ1 is primarily upregulated during the proliferative, neurogenic response of Müller cells (Lenkowski et al., 2013). Contrarily, TGFβ3 collaborates with Notch signaling to inhibit retinal regeneration following retinal injury in zebrafish (Lee et al., 2020).

We performed a cross-species analysis comparing animal models with fully regenerative capacity (zebrafish) and models with minimal/absent regeneration, which showed that TGFβ isoforms play a different role in retinal tissue repair, underscoring the pleiotropic nature of TGFβ action (Conedera et al., 2021b). Furthermore, canonical Smad signaling was upregulated during the proliferative, neurogenic response of Müller cells (Lenkowski et al., 2013). Contrarily, TGFβ3 collaborates with Notch signaling to inhibit retinal regeneration following retinal injury in zebrafish (Lee et al., 2020).

Recently, evidence demonstrated that the regulation of TGFβ depends on its interaction with other pathways. Essential for tissue repair mechanisms in diverse organs (e.g., kidney, liver, and heart) is also the Notch pathway. Several physiological and pathological processes are regulated by Notch concomitant with TGFβ expression, thus setting the stage for a possible interplay between the two pathways. We determined the importance of TGFβ/Notch during laser-induced injury response of Müller cells by cross-species comparison. We showed that TGFβ/Notch interplay in a Smad3-dependent manner and triggers cycle arrest of Müller cells. This results in an unsuccessful reprogramming during reactive gliosis in mice, and Smad3 inhibition boosts the limited regenerative potential of murine Müller cells. Moreover, our findings suggest Müller cells shift towards an epithelial lineage (Müller cell - epithelial transition [MC-ET]) during reactive gliosis in mammals providing novel insights into the remodeling mechanism of retinal degeneration. In our model, we also determined the activation of gliotic markers in zebrafish, which suggests transient gliosis and its regression precedes the photoreceptors’ regeneration. However, reactive gliosis persists in murine Müller cells throughout the injury response. Currently, it is unclear how the regenerative activity of Müller cells exacerbates chronically the injury response, which ultimately leads to glial scar formation. The regenerative capacity of Müller cells is essential for tissue repair and protection with their exit from quiescence in response to injury. In both zebrafish and mice, Müller cells re-entered into the cell cycle in response to injury. In zebrafish, the signal returned to baseline in the restored retina, which supports the hypothesis of a transient reactive gliosis. In mice, Müller cells abnormally proliferated and that resulted in an arrested double-strand break repair response. Thus, we identify the initial expression of marker proteins in both animals in our injury model, suggesting the possibility of Müller cells behave as progenitor/stem cells (Sambrook et al., 2021). Importantly, both TGFβ1 and Notch are expressed in mouse Müller cells undergoing endothelial-like changes. The unsuccessful reprogramming during chronic gliosis illustrates that murine Müller cells cannot ensure proper gliosis repair/de-differentiation during the injury response leading to arrested re-entry into mitosis. That induced double-strand breaks with the subsequent acquisition of epithelial features by Müller cells during glial scar formation. During epithelial transformation, characterized by DDR, Notch1/2 was upregulated in response to injury in mice only. The simultaneous activation of TGFβ and Notch in murine Müller cells indicates their combined action during chronic gliosis after laser-induced retinal degeneration. Interestingly, a gliotic reaction occurred in our mouse model, which illustrates that TGFβ/Notch interplay in a Smad3-dependent manner and triggers cycle arrest of Müller cells (Lee et al., 2020). These data link MC-ET to TGFβ/Notch during chronic reactive gliosis in humans comparable to retinal injury response in mice (Figure 1A).

The impact of arrested re-entry into mitosis on Müller cell differentiation was investigated in our model. TGFβ or Notch inhibition on reactive gliosis in mice was investigated upon laser-induced retinal injury in our retina degeneration model, which ultimately leads to glial scar formation. We showed that TGFβ1 and TGFβ2 could trigger DDR, in line with the arrested re-entry into mitosis in mice.
Furthermore, we associated DAPT with fibrotic-like outcomes in Müller cells at the expense of their mesenchymal-neuronal potential (Figure 1B). These data propose MC-ET as a repair mechanism following cell-cycle arrest. Pirfenidone showed the potential to regress the TGFβ-induced MC-ET, and TGFβ inhibition trapped Müller cells into quiescence even after injury in mice (Figure 1B). However, targeting TGFβ may affect other physiological mechanisms, owing to its pleiotropic nature. Our data revealed that TGFβ might act upstream of Notch. The Notch pathway was inhibited by DAPT (inhibitor of y-secretase) to preserve TGFβ and prevent MC-ET-associated fibrosis after laser-induced retinal degeneration. Transient exposure to DAPT confirmed the link between TGFβ and Notch. However, pro-fibrotic TGFβ response seems independent of Notch inhibition. Inhibitions of either TGFβ or Notch were unsuccessful in stimulating further improvements during injury response in mice (Figure 1B). Finally, we tested if the combined action of TGFβ/Notch mediates MC-ET. TGFβ cooperates with Notch in a Smad3-dependent manner, and both synergistic and antagonistic effects of TGFβ/Notch interplay have been reported (Wang et al., 2017). Smad3 was suppressed using a selective inhibitor of Smad3 (SIS3) during early injury response, MC cell-cycle arrest, and MC-ET in mice (24-hour treatment). TGFβ3 favored a mesenchymal response in Müller cells at the expense of their epithelial transformation via TGFβ1/2 after laser-induced retinal degeneration (Figure 1B). Based on this promising data, we extend SIS3 treatment throughout the experiment. SIS3 treatment showed that murine Müller cells are capable of exiting their quiescence state, a critical step toward regeneration. Thereby, SIS3 showed the potential to modulate MC-ET-associated fibrosis and reduce the glial scar in vivo by stimulating the de-differentiation of murine Müller cells.

In our opinion, reactive gliosis, Müller cell-epithelial transition, and ensuing fibrosis are the main characteristics of retinal degeneration. Thus, alleviating the detrimental effects of glial scar formation via modulation of these players might enable retinal regeneration in mammals.

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