



Insights into Muller Glia proliferation: A Look Through Different Species

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Untreatable blindness is majorly caused by loss of the light sensitive photoreceptor cells. Studies to this aspect has been done in various species. Based on the developmental ability and functioning of signaling cascades, animals behave differently to the loss of photoreceptor cells. Lower vertebrates, like frogs and fish, have exceptional capacity for repairing the retina after damage but this regenerative potential is very limited to mammals. Studies in fish and frogs have revealed key pathways involved in the retina repair mechanism. The prominent among them is Muller glia cells-based reactivation, leading the cells to re-enter the cell cycle and develop new neurons like photoreceptor cells.

Sight for any living visual animal is considered as the most important sense, where retina derives potential sensory input to the brain (Jayakody et al., 2015). The mammalian and non-mammalian species possess similar retinal architecture various neurons and two light sensitive photoreceptor cell types (Karl and Reh, 2010). Retinal degeneration could lead to retinitis pigmentosa, diabetic retinopathy and age-related macular degeneration. As significant advances have raised hopes of therapy for some forms of inherited blindness, the majority of currently available treatments aim to slow the progression of degeneration. Till date, there are no treatments available that can reverse the loss of vision occurred due to photoreceptor cell death. To this context, understanding self-repair mechanism in species equipped with regenerative properties will help in developing therapeutic strategies to trigger such process in the patients suffering from retinal diseases. (Langhe et al., 2017).

Different therapeutic strategies considered such as, photoreceptor replacement by stem cell-derived photoreceptor and/or RPE transplantations (Jayakody et al., 2015; Aghaizu et al., 2017). Photoreceptor cells obtained from 3D cultures of pluripotential stem cells (PSCs) from both murine and human sources can also be used (Kruczek et al., 2017; Gonzalez-Cordero et al., 2017). Interestingly material transfer strategy could also be useful. This involves the robust exchange of material (RNA and/or protein) between donor and host photoreceptors, rendering the host acceptor photoreceptor cells functional (Pearson et al., 2016). But still all these therapeutic techniques are very expensive, time consuming and still more research is required in this aspect.

Endogenous self-repair mechanism in patients' earlier disease stage, by recruiting cells within the retina that has stem cell-like properties to develop new neurons following



retinal injury. Till date lower vertebrates have played important role in the studying regenerative process. Amphibians possess exceptional capacity repairing the damaged retina because their eyes continue to grow throughout their life (Karl and Reh, 2010). Endogenous repair mechanism could take place via different cell populations depending on the extent and type of the retinal damage. One such cellular source with potential to mediate retinal repair includes Muller glia (Hamon et al., 2016).

As major glial cells type, Muller glia spans through entire thickness of the retina. Muller glia are one of the important types of support cell in the retina, which provides structural and metabolic support to the neurons. Hence the action of Muller glia results in the function and survival of the retinal cells (Bringmann et al., 2006). As they are based throughout the apico-basal extent of the retina, due to which they sense the retinal damage and react accordingly (Langhe and Pearson, 2020). The response of the Muller glia depends on the type and the extent or the model of the injury. Due to their quiescent nature different injury models and paradigms have been used to study the regenerative potential. Few important injury models that can be highlighted are needle poke injury, laser ablation, light exposure, treatment with Metronidazole, injections of Ouabain, and N-Methyl-D-aspartic acid (Ail and Perron, 2017). Various fish species, *Xenopus* frogs, chicks and mammals such as mice are utilized to study retina regeneration.

In *Xenopus laevis* frogs, following either needle poke injury or nitroreductase-mediated photoreceptor ablation, the MG are capable of re-entering the cell cycle and generating new photoreceptors. This is age-dependent process, which is more effective in pre-metamorphic tadpoles and adult frogs, in comparison to young tadpole stages, which is observed in above two different injury models (Langhe et al., 2017).

Following injury in the zebrafish retina, the pro-inflammatory cytokine tumour necrosis factor alpha (TNF α) is produced by dying retinal neurons and has been shown to trigger Muller Glia dedifferentiation and proliferation. This phenomenon is controlled through *Stat3* and *Ascl1a* signalling (Nelson et al., 2013). Growth factors play important role in deciding fate of Muller glial cells. Various growth factors such as insulin growth factor-1, HB-EGF, FGF2 trigger Muller glia de-differentiation and proliferation through the MAPK pathway (Wan et al., 2014).

In the MNU-induced injury model, inhibition of transforming growth factor beta (TGF β) signalling pathway results in increased Muller glia proliferation (Tappeiner et al., 2016). *Sox,2* a transcription factor is necessary, and ectopic expression of *Sox2* is necessary to trigger Muller glia proliferation (Gorsuch et al., 2017). *Sox2* is required for high expression of *Ascl1a* through the induction of Lin28a-dependent repression of let-7 miRNA biogenesis that is necessary for amplification of Muller glia. In Medaka fish, induction of a pro-neural transcription factor, *Atoh7* is enough for Muller glia to guide them to reenter the cell cycle and proliferate new neurons. Interestingly, this occurs even



in the absence of injury. The interplay between *Ascl1* pathway, a downstream target and *Wnt* signalling plays crucial role in proliferation. Notch signalling is also downstream of *Ascl1*, where *Ascl1* coordinates the expression of several genes encoding Notch ligand (Nelson et al., 2009). In the uninjured zebrafish, *Ascl1/Lin28*-mediated MG proliferation is increased by inhibiting Notch signalling (Elsaedi et al., 2018).

Generally adult birds lack retinal regenerative capacity following injury. But few studies in postnatal chick exhibits promising regenerative ability following as N-methyl-D-aspartic acid induced damage, where in few new neurons are generated (Fischer and Reh, 2001). This is stimulated by Notch signalling pathway. The action of Hedgehog signalling on MG proliferation is increased by FGF2/MAPK signalling, as is *Wnt/β-catenin*, the activation of which is required for the formation of Muller glia derived progenitors in chick (Gallina et al., 2016).

In contrast mammalian Muller glia has limited regenerative potential as compared to frogs, fish and postnatal chicks. But outlook changed when a study was performed by administrating NMDA and later combining this with the administration of growth factors such as FGF1, insulin and EGF. A limited number of amacrine cells were generated (Karl et al., 2008). Various signalling pathways such as *Wnt*, *Ascl1*, EGF and *Shh* were reported to play role in Muller glia (Langhe and Pearson, 2020). Only young mice showed generation of bipolar and amacrine cells following the NMDA induced injury (Ueki et al., 2015).

Another study on adult mice with NMDA induced retinal injury exhibited that combined administration of trichostatin-A (TSA), a histone deacetylase inhibitor, with *Ascl1* leads to increased Muller glia proliferation (Jorstad et al., 2017). *Wnt/β-catenin* signaling is upregulated and involved in limited Muller glia proliferative response after laser induced retinal injury in mice. In adult mice, administration *β-catenin* is enough for inducing proliferation. As *β-catenin* binds *Lin28*, which signifies the interplay of *Wnt* and *Lin28/Let7* pathways in the proliferation (Yao et al., 2016).

To conclude, even though amphibians and birds models exhibit promising Muller glia proliferation leading to retinal repair. Still more research is yet to be done on mammalian models to implement endogenous repair mechanism via Muller glia proliferation, which could ultimately be utilized for the therapeutic treatment.



References

1. Aghaizu ND, Kruczek K, Gonzalez-Cordero A, Ali RR, Pearson RA. Pluripotent stem cells and their utility in treating photoreceptor degenerations. In: *Progress in Brain Research*. Vol 231; 2017:191-223.
2. Ail D, Perron M. Retinal Degeneration and Regeneration—Lessons From Fishes and Amphibians. *Curr Pathobiol Rep*. 2017;5(1):67-78.
3. Bringmann A, Pannicke T, Grosche J, Francke M, Wiedemann P, Skatchkov SN, Osborne NN, Reichenbach A. 2006. Müller cells in the healthy and diseased retina. *Prog Retin Eye Res* 25:397–424
4. Elsaedi F, Macpherson P, Mills EA, Jui J, Flannery JG, Goldman D. Notch Suppression Collaborates with Ascl1 and Lin28 to Unleash a Regenerative Response in Fish Retina, But Not in Mice. *J Neurosci*. 2018;38(9):2246-2261.
5. Fischer AJ, Reh TA. Müller glia are a potential source of neural regeneration in the postnatal chicken retina. *Nat Neurosci*. 2001;4(3):247-252.
6. Gallina D, Palazzo I, Steffenson L, Todd L, Fischer AJ. Wnt/ β -catenin-signaling and the formation of Müller glia-derived progenitors in the chick retina. *Dev Neurobiol*. 2016;76(9):983-1002.
7. Gonzalez-Cordero A, Kruczek K, Naeem A, et al. Recapitulation of Human Retinal Development from Human Pluripotent Stem Cells Generates Transplantable Populations of Cone Photoreceptors. *Stem Cell Reports*. 2017;9(3):820-837.
8. Gorsuch RA, Lahne M, Yarka CE, Petravick ME, Li J, Hyde DR. Sox2 regulates Müller glia reprogramming and proliferation in the regenerating zebrafish retina via Lin28 and Ascl1a. *Exp Eye Res*. 2017;161:174-192.
9. Hamon A, Roger JE, Yang XJ, Perron M. Müller glial cell-dependent regeneration of the neural retina: An overview across vertebrate model systems. *Dev Dyn*. 2016;245(7):727-738.
10. Jayakody SA, Gonzalez-Cordero A, Ali RR, Pearson RA. Cellular strategies for retinal repair by photoreceptor replacement. *Prog Retin Eye Res*. 2015;46:31-66.
11. Jorstad NL, Wilken MS, Grimes WN, et al. Stimulation of functional neuronal regeneration from Müller glia in adult mice. *Nature*. 2017;548(7665):103-107.
12. Karl MO, Hayes S, Nelson BR, Tan K, Buckingham B, Reh TA. Stimulation of neural regeneration in the mouse retina. *Proc Natl Acad Sci*. 2008;105(49):19508-19513.
13. Karl MO, Reh TA. Regenerative medicine for retinal diseases: activating endogenous repair mechanisms. *Trends Mol Med*. 2010;16(4):193-202.
14. Kruczek K, Gonzalez-Cordero A, Goh D, et al. Differentiation and Transplantation of Embryonic Stem Cell-Derived Cone Photoreceptors into a Mouse Model of End-Stage Retinal Degeneration. *Stem Cell Reports*. 2017;8(6):1659-1674.



15. Langhe R, Chesneau A, Colozza G, et al. Müller glial cell reactivation in *Xenopus* models of retinal degeneration. *Glia*. 2017;65(8):1333-1349.
16. Langhe R, Pearson RA. Rebuilding the Retina: Prospects for Muller Glial-mediated self-repair. *Curr Eye Res*. 2020;45(3):349-360.
17. Nelson BR, Hartman BH, Ray CA, Hayashi T, Bermingham-McDonogh O, Reh TA. Acheate-scute like 1 (*Ascl1*) is required for normal delta-like (*Dll*) gene expression and notch signaling during retinal development. *Dev Dyn*. 2009;238(9):2163-2178.
18. Nelson CM, Ackerman KM, O'Hayer P, Bailey TJ, Gorsuch RA, Hyde DR. Tumor Necrosis Factor-Alpha Is Produced by Dying Retinal Neurons and Is Required for Muller Glia Proliferation during Zebrafish Retinal Regeneration. *J Neurosci*. 2013;33(15):6524-6539.
19. Pearson RA, Gonzalez-Cordero A, West EL, et al. Donor and host photoreceptors engage in material transfer following transplantation of post-mitotic photoreceptor precursors. *Nat Commun*. 2016;7(1):13029.
20. Tappeiner C, Maurer E, Sallin P, Bise T, Enzmann V, Tschopp M. Inhibition of the TGF β Pathway Enhances Retinal Regeneration in Adult Zebrafish. Thummel R, ed. *PLoS One*. 2016;11(11):e0167073.
21. Ueki Y, Wilken MS, Cox KE, et al. Transgenic expression of the proneural transcription factor *Ascl1* in Müller glia stimulates retinal regeneration in young mice. *Proc Natl Acad Sci*. 2015;112(44):13717-13722.
22. Wan J, Zhao X-F, Vojtek A, Goldman D. Retinal Injury, Growth Factors, and Cytokines Converge on β -Catenin and pStat3 Signaling to Stimulate Retina Regeneration. *Cell Rep*. 2014;9(1):285-297.
23. Yao K, Qiu S, Tian L, et al. Wnt Regulates Proliferation and Neurogenic Potential of Müller Glial Cells via a *Lin28/let-7* miRNA-Dependent Pathway in Adult Mammalian Retinas. *Cell Rep*. 2016;17(1):165-178.