TITLE: Unlocking the potential for endogenous repair to restore sight

AUTHORS: Rachael A. Pearson[1], Robin R. Ali[1,2]

AFFILIATIONS: [1]University College London Institute of Ophthalmology, 11-43 Bath Street, London, EC1V 9EL, UK. [2]Kellogg Eye Center, University of Michigan, 1000 Wall St., Ann Arbor, Michigan 48105, USA

CORRESPONDENCE TO: rachael.pearson@ucl.ac.uk, +44(0)2076084022

The eye often leads the way in the development of novel strategies for treating neurodegenerative disorders. Here, we highlight an exciting new study (Yao et al., 2018) examining the potential for reactivating endogenous repair mechanisms and enabling the mammalian retina to repair itself.

Photoreceptors are terminally differentiated neurons, which once lost are not replaced. In industrialized countries, conditions such as retinitis pigmentosa, age-related macular degeneration (AMD) and diabetic retinopathy, which are characterized by photoreceptor degeneration, are the major causes of registered blindness. At present, there are few effective therapies and the majority attempt to slow vision loss. Regenerative therapies, by contrast, aim to reverse vision loss by replacing the dying cells. Over the past decade this approach has seen significant progress with the development of transplantation of stem cell-derived photoreceptors and several groups are now moving towards clinical trials. At present, however, the process of producing stem cell-derived retinal cells *in vitro* is costly and time-consuming, and there may be immune rejection. An attractive, but unproven, alternative is to try to unlock the potential for endogenous regeneration.

In some non-mammalian vertebrates, like zebrafish, retinal injury evokes a highly efficient endogenous repair mechanism. In response to damage, the radial glia of the retina, Müller cells, de-differentiate to a stem cell-like state. The regenerative process proceeds with asymmetric cell division to regenerate the Müller cell itself and produce a daughter cell, which undergoes subsequent rounds of division to replace the cells that are lost. By contrast, the mammalian retina lacks the capacity for self-repair and the Müller glia respond to damage by undergoing reactive gliosis. Although initially neuroprotective, persistent gliosis causes scarring and neuronal loss. Zebrafish show similar changes in the early stages post-injury, but the response then transitions to one of Müller cell proliferation.

This difference in the regenerative potential of non-mammalian and mammalian Müller cells has been the subject of significant investigation over the past decade. Numerous groups have sought to identify the signaling pathways responsible for Müller cell-mediated regeneration in zebrafish, and other models, with the hope of subsequently reactivating them in the

mammalian retina. A key event is the re-expression of the proneural gene *Ascl1* and subsequent induction of Lin28, a pluripotency mRNA-binding protein (Ramachandran et al., 2010). Lin28 suppresses the actions of *let-7*, which otherwise inhibits the proliferative response of Müller glia. Recently, *Sox2* (Gorsuch et al., 2017) and *Atoh7* (Lust et al., 2016) have also been shown to be re-expressed in Müller cells after injury and are sufficient to drive their proliferation to generate new neurons. *Sox2* is able to induce both *Ascl1* and *Atoh7*.

Importantly, *Ascl1* is not up-regulated significantly after injury in mice, which has led to the hypothesis that this pathway is key to differences in the regenerative capabilities of Müller glia in mammals and other species. In support of this hypothesis, Reh and colleagues have shown that gene transfer of *Ascl1* is sufficient to activate a neurogenic program in adult mammalian Müller glia both *in vitro* and in the intact retina, but only if combined with injury, and the response was largely restricted to young mice. They proposed that this was due to limitations in the chromatin accessibility of target genes and the same team recently reported that combining the over-expression of *Ascl1* with application of a histone deactylase inhibitor in damaged adult mouse retinae enabled a subset of Müller glia to proliferate and generate inner retinal neurons (Jorstadt et al., 2017).

In zebrafish, Wnt and its downstream target *β-catenin* also play an integral role in the injury-induced regenerative response. Remarkably, in the uninjured retina, inhibition of glycogen synthase kinase-3β (GSK-3β), which mimics the activation of Wnt, was sufficient to stimulate Müller glial dedifferentiation and the formation of multipotent retinal progenitors that are capable of generating all retinal cell types. *Ascl1* was found to contribute to the multipotential character of these progenitors. Induction of *Ascl1* also suppressed expression of the Wnt signalling inhibitor, *Dkk*, and induced expression of *Wnt* (Ramachandran et al., 2011), demonstrating the interplay of these pathways.

Wnt signalling has previously been implicated in the limited Müller cell proliferation that occurs following injury in the murine retina (Osakada et al., 2007) and a previous study by Chen and colleagues indicated that gene transfer of ***β-catenin*** is sufficient to activate both Wnt and the Lin28/*let-7* pathway to induce Müller glial proliferation in adult mice *without* injury (Yao et al., 2016). In their most recent study, Chen and colleagues build on these findings, describing a multi-step process including induction of mammalian Müller glia dedifferentiation using virus-mediated over-expression of ***β***-catenin, which precedes a single round of Müller cell division. This is followed two weeks later by the introduction, by viruses, of the photoreceptor transcription factors, *Crx, Otx2* and *Nrl*, with co-expression of all three being necessary for the generation of new rod photoreceptors.

These two studies by Chen and colleagues (Yao et al., 2016; 2018) highlight an important difference with previous investigations where the wildtype murine retina appeared to lack a significant regenerative response. Indeed, even overexpression of key reprogramming factors such as *Ascl1* failed to yield significant Müller cell proliferation in the absence of injury. When coupled with injury, overexpression of *Ascl1* has typically been associated with the generation of inner retinal neurons, while production of photoreceptors has been limited. It will be very interesting to determine whether ectopic expression of photoreceptor transcription factors combined with *Ascl1*-mediated induction of Müller glial dedifferentiation can similarly lead to the generation of new photoreceptors, or whether other pathways downstream of *Wnt/β-catenin,* but not *Ascl1/Lin28,* are required for this more comprehensive repair response. Given the importance of cone photoreceptors on human vision, it will also be crucial to determine whether Müller cells can be induced to generate cones.

Definitive proof of the fate acquired by newly-generated Müller cell-derived neurons has been particularly problematic in the field to date; the majority of studies have used the incorporation of markers such as BrdU to identify newly-generated cells and classification of fate has typically relied on retinal positioning and co-labelling with cell-type specific markers. Indeed,

the majority of the experiments in this most recent study by Chen and colleagues rely on promoter-driven fluorescent reporters, which also have their limitations for fate-tracing; cautionary lessons may be drawn from the photoreceptor transplantation field, where it had previously been understood that transplanted donor photoreceptors, labelled with promoter-driven fluorescent reporters, became incorporated into the adult wildtype retina. Recently however, we, and others, have shown that most of the apparently incorporated cells are in fact host cells that have acquired proteins and/or RNA from the donor cells, via a process we termed "material transfer" (Pearson et al., 2016), the underlying cellular mechanisms for which are currently unknown. Chen and colleagues verify some of their key findings using Cre-lox lineage tracing. This is an important tool and should be used routinely, but caution in interpretation of experiments involving reporters is still prudent in light of the discovery of material transfer.

In a remarkable extension to their study, Yao et al. (2018) induced Müller glia regeneration in the *Gnat1*$^{-/-}$ mouse, in which rod photoreceptors are non-responsive. They supplemented *Gnat1* back into the Müller cells (to rescue new photoreceptor progeny) and reported a significant rescue of light-induced retinal activity. Methodologically, this is an astonishing feat, since each re-programmed Müller cell must be transduced by 7 different viruses in order to complete this step-wise regeneration programme. These findings appear to demonstrate an important proof of principle but going forward we must consider whether such regenerative potential is sustained in the degenerating retinal environment. To date, we have remarkably little understanding regarding the potential for Müller cell-mediated regeneration in the dystrophic mammalian retina. Müller glia behave very differently in response to acute, versus chronic, injury. Indeed, gliosis is a key example where the type of injury yields markedly different outcomes in terms of the extent and duration of the response. Notably, retinal gliosis is minimal in fish. Most reports to date have focussed either on gene transfer to Müller cells in the uninjured retina or have combined it with acute injury. How Müller glia respond to chronic loss of photoreceptors, as occurs in inherited retinal dystrophies and AMD, is less well understood, but we may expect the responses to be different: In an early study by Takahashi and colleagues, they also sought to utilise the *Wnt/β-catenin* pathway in the rapidly degenerating *rd1* mouse and found that while Wnt3a could activate β-catenin and induce Müller cell proliferation in postnatal (P)12 retinal explants, no regeneration was seen in P21 retinae (Osakada et al., 2007).

An important question facing both transplantation and endogenous repair is how 'normal' can we expect the newly generated circuitry, and resulting visual function, to be? The rescue of visual function in the non-degenerative *Gnat1*$^{-/-}$ mouse (Yao et al., 2018) is striking. In the absence of photoreceptor degeneration, the newly-generated cells are, presumably, supernumerary. Do they piggy-back into the existing circuitry or cause remodelling of existing connections to occur? The latter is, perhaps, easy to envisage in the degenerating retina, where post-synaptic remodelling is already taking place, but it is intriguing to consider how this process occurs in the uninjured retina. Studies in the zebrafish have indicated that regenerated bipolar cells form most of their stereotypical post-synaptic connections but do not perfectly rewire with photoreceptors (D'Orazi et al., 2016), suggesting that a complete recapitulation of normal retinal development would be surprising. Nonetheless, the regenerated retina recovers visually-evoked behaviours, indicating that sufficient wiring can be re-established. Careful analysis of the connectome resulting from the generation and integration of new neurons, particularly where no host photoreceptor cells remain, will be key. Indeed, the ultimate validation of these striking new findings will be the Müller glial-mediated rescue of visual function in end-stage disease, where no host photoreceptors could potentially contribute to any observed rescue. Such results will, of course, also be of the utmost importance as we try to realise the clinical application of endogenous retinal repair for the treatment of blindness.

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