

● INVITED REVIEW

Endogenous retinal neural stem cell reprogramming for neuronal regeneration

Romain Madelaine^{1,*}, Philippe Mourrain^{1,2,*}

1 Stanford Center for Sleep Sciences and Medicine, Department of Psychiatry and Behavioral Sciences, Stanford University, Stanford, CA, USA
2 INSERM 1024, Ecole Normale Supérieure, Paris, France

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Abstract

In humans, optic nerve injuries and associated neurodegenerative diseases are often followed by permanent vision loss. Consequently, an important challenge is to develop safe and effective methods to replace retinal neurons and thereby restore neuronal functions and vision. Identifying cellular and molecular mechanisms allowing to replace damaged neurons is a major goal for basic and translational research in regenerative medicine. Contrary to mammals, the zebrafish has the capacity to fully regenerate entire parts of the nervous system, including retina. This regenerative process depends on endogenous retinal neural stem cells, the Müller glial cells. Following injury, zebrafish Müller cells go back into cell cycle to proliferate and generate new neurons, while mammalian Müller cells undergo reactive gliosis. Recently, transcription factors and microRNAs have been identified to control the formation of new neurons derived from zebrafish and mammalian Müller cells, indicating that cellular reprogramming can be an efficient strategy to regenerate human retinal neurons. Here we discuss recent insights into the use of endogenous neural stem cell reprogramming for neuronal regeneration, differences between zebrafish and mammalian Müller cells, and the need to pursue the identification and characterization of new molecular factors with an instructive and potent function in order to develop therapeutic strategies for eye diseases.

Key Words: neuronal regeneration; retina; Müller glial cells; neural stem cell reprogramming; *achaete-scute* homolog 1; *microRNA-9*; *Tlx*; *Onecut*

Introduction

In humans, retinal injury or neurodegenerative diseases are often followed by permanent loss of vision. Outer retinal diseases such as macular degeneration lead to the death of the photoreceptor cells and adjacent retinal pigment epithelium. In the inner retina, loss of retinal ganglion cells (RGCs) like in glaucoma and optic nerve degeneration are the most common diseases affecting vision. Unfortunately, loss of photoreceptor cells or RGCs is irreversible, and often leads to blindness. Today, identifying efficient approaches to replace lost retinal neurons and restore functional vision is a major challenge for basic and translational research in regenerative medicine.

Stem Cells Transplantation for Retinal Repair: Hopeful but Risky Strategy

For patients who have lost retinal neurons, we must identify safe and effective methods to replace these cells. Stem cells transplantation is currently a popular approach as these cells have the ability to self-renew for a long period of time and the capacity to differentiate into multiple cell types. Neural stem cells (NSCs) are a specific type of stem cells with the ability to differentiate into neurons and glial cells (Gage, 2000), and can potentially be used for cellular regenerative therapy following the loss of neurons. In human retinal diseases, stem cell-based therapies provide a new strategy for neuropathies as exogenous neurons derived from stem cells have the potential capacity to replace neurons that have been lost and restore visual

function. But retinal neurons replacement from exogenous cells is a complex process, as it requires both local retinal integration as well as optic nerve regeneration and correct connection to the pre-existing neuronal network like in the case of RGCs. Furthermore, with all exogenous cell transplantation, the risk associated with cell transfer is that transplanted stem cells and newly differentiated neurons may be recognized by the host as “non-physiologic” and rejected by the immune system. An alternative strategy is to use autologous stem cells, but again this procedure is not without a risk as it is complicated, if not impossible, to precisely control the cell fate following stem cells transplantation. Recently, a case report highlighted the need of tight control in stem cell transplantation procedures as three patients suffering from age-related macular degeneration who received autologous adipose tissue-derived stem cells showed blindness after 1 year following the injection (Kuriyan et al., 2017). A promising approach to bypass the problems coming with stem cells transplantation would be to recruit endogenous NSCs and to reprogram them to promote neuronal regeneration. This strategy would allow to generate new retinal neurons from endogenous stem cells, and can potentially be used to control the neuronal regeneration in all species harboring retinal NSCs, notably in human.

Endogenous NSC Reprogramming Restores Vision in the Zebrafish Retina

The endogenous regenerative process of the retina after injury occurs with high efficiency in some non-mammalian

*Correspondence to:

Romain Madelaine or
Philippe Mourrain,
rom1983@stanford.edu or
mourrain@stanford.edu.

orcid:

0000-0002-6622-415X
(Romain Madelaine)

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vertebrates, notably in zebrafish (Lenkowski and Raymond, 2014). Indeed, following retinal injury and retinal neurons loss, the zebrafish NSCs of the retina (Müller glial cells) go back into the cell cycle to generate proliferative neural progenitors allowing the regeneration of all the retinal neuron subtypes and the restoration of functional vision. The Müller cells are radial glial cells and express the glial fibrillary acidic protein and glutamine synthase markers. The radial morphology and expression of glial markers make these cells similar to embryonic radial glial cells used as progenitor cells in the central nervous system of developing mammals (Malatesta and Gotz, 2013). These observations suggest that radial glial cells are endogenous NSCs in the brain and retina. Under physiological conditions, NSCs are relatively quiescent to avoid the precocious depletion of the NSCs pool. Consequently, the use of endogenous NSCs for neuronal regeneration will require these cells to exit from their latent state to undergo proliferation and neuronal differentiation. Few of the molecular factors controlling Müller cells proliferation and differentiation have been identified in zebrafish. To date, it appears that a key event in the retinal NSCs reprogramming after injury is the re-expression of proneural transcription factor such as achaete-scute homolog 1a (*Ascl1a*) and the subsequent induction of *lin-28*, a pluripotency mRNA binding protein (Ramachandran et al., 2010). After injury, *ascl1a* expression is detected in proliferative neural progenitors and its inhibition limits the capacity of retinal neurons regeneration after injury. Recently, two other transcription factors, sex determining region Y-box 2 (*Sox2*) (Gorsuch et al., 2017) and atonal homolog 7 (*Atoh7*) (Lust et al., 2016), have been identified to be re-expressed in Müller cells after injury. In addition, *Sox2* and *Atoh7* are sufficient to drive quiescent Müller cells back in proliferation to generate new retinal neurons, indicating that they carry instructive functions promoting cell proliferation and neuronal differentiation. Interestingly, *Sox2* has the capacity to induce *Ascl1a* and *Atoh7* expression which themselves are able to activate *lin-28* transcription. While their timing of induction following *Sox2* expression indicates that *ascl1a* is expressed before *atoh7*, it still remains to be determined whether *atoh7* induction is downstream of *Ascl1a* or if they both act in parallel pathways to control *lin-28* expression and cellular reprogramming. Altogether, these observations indicate that *Sox2-Ascl1a/Atoh7-Lin-28* is a critical transcriptional cascade controlling NSCs reprogramming and neuronal regeneration process after injury in the retina (Figure 1).

Limited Capacity of Retinal Stem Cells Dependent Neuronal Regeneration in Mammals

While Müller cells are found in all vertebrates, mammalian Müller cells do not respond to injury by undergoing a regenerative process but instead they experience reactive gliosis inhibiting the regeneration process. Nevertheless, mammalian Müller cells have demonstrated potential to act as progenitors of photoreceptor cells (Giannelli et al., 2011) or bipolar neurons (Pollak et al., 2013) *in vitro*, demonstrat-

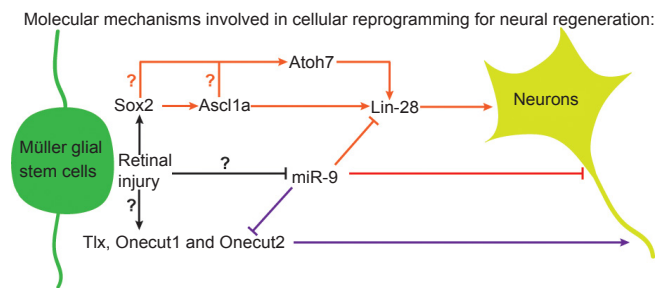


Figure 1 Schematic representation of molecular pathways involved in retinal neuron regeneration from endogenous neural stem cells. Known positive and negative regulations are indicated. miR-9 links orange and purple transcriptional cascades *via* the direct inhibition of Lin-28 mRNA binding protein and Tlx/Onecut transcription factors expression. Atoh7: Atonal homolog 7; Sox2: sex determining region Y-box 2; Ascl1a: achaete-scute homolog 1a; miR-9: microRNA-9; Tlx: tailless box vertebrate homologue.

ing their NSCs properties. Further, the Reh lab has recently demonstrated that exogenous expression of the proneural transcription factor *Ascl1* has the ability to promote mammalian Müller cells proliferation to generate amacrine, bipolar and photoreceptor neurons after retinal injury (Ueki et al., 2015). However, this regenerative process is restricted to young mice because chromatin accessibility of target genes is limited in adults. But combining *Ascl1* expression with a histone deacetylase inhibitor can bypass this limitation and increase the generation of new neurons in the adult retina (Jorstad et al., 2017). These regenerated neurons integrate the existing neuronal network and are able to respond to light, indicating their functionality and suggesting that they can likely be used to restore vision. While these results are extremely promising, the regenerative response is still limited, likely because the proliferative capacity of mammalian Müller cells is low compared to their zebrafish counterparts. This observation indicates that additional factors, that are not downstream of *Ascl1*, are required to increase the mitotic activity of the retinal NSCs in order to fully regenerate human retinal neurons and restore vision.

Future Directions

As discussed above, a promising approach to induce retina repair is to reprogram retinal NSCs, the Müller cells, to promote the formation of new neurons. We recently uncovered the expression of microRNA-9 (miR-9) in zebrafish Müller cells and identified miR-9 as a critical factor controlling retinal NSCs proliferation and fate (Madelaine et al., 2017). We found that miR-9 depletion increases the number of neural progenitors and neurons in the zebrafish retina. Interestingly, *lin-28*, which is necessary to promote the proliferation of retinal NSCs after injury, is a target of miR-9 (La Torre et al., 2013). The above observations suggest that miR-9 acts as a negative regulator of the *Sox2-Ascl1a/Atoh7-Lin-28* pathway to prevent Müller cells proliferation. Consequently, we reasoned that miR-9 downstream targets are likely positively regulating NSCs proliferation and differentiation. We identified miR-9 direct targets, *TLX* and *ONECUT* mRNAs, and showed that expression of *TLX* or *ONECUT* transcription factors in human or zebrafish NSCs promote their differ-

entiation into neurons (Madelaine et al., 2017). Altogether, these observations support a very promising role for the miR-9-*TLX-ONECUT* pathway in the reprogramming of endogenous Müller cells into functional retinal neurons. As therapeutic application to restore vision will require a precise control of the cell proliferation and neuronal fate, it will be important to understand how this pathway integrates with the Sox2-Ascl1a/Atoh7-Lin-28 cascade (Figure 1), and to pursue the identification of the factors controlling Müller cells proliferation and differentiation.

As the main limitation in the neuronal regeneration field is our ability to reprogram endogenous NSCs *in vivo* to allow the re-entry of these cells into the cell cycle to generate new neurons, it is indeed necessary to identify new factors increasing the efficiency of the regenerative response. For example, reprogramming of mouse Müller cells with Ascl1 only induces a moderate regeneration of bipolar cells (Jorstad et al., 2017). It is likely that additional factors controlling Müller cells capacity to go back in the cell cycle and to actively proliferate are not under the regulation of Ascl1. Indeed, it has been shown in zebrafish that growth factors and cytokines are produced by Müller cells following retinal injury, and that these factors can also promote their proliferation without injury (Wan et al., 2014). Because mammalian Müller cells have a much more limited capability to regenerate retinal neurons compared to their zebrafish counterpart, it is critical to understand what factors allow zebrafish retinal tissue to regenerate so efficiently to then transfer such knowledge to human retina regenerative medicine. In the coming years, the quick emergence of powerful single cell RNA sequencing techniques should allow us to identify new candidate genes involved in Müller cells proliferation and differentiation. Genes that are differentially expressed in zebrafish Müller cells after injury, but also differentially expressed between zebrafish and mouse Müller cells, are of particular interest to be tested for retinal NSCs reprogramming in mammals. The identification of such factors would greatly increase our capacity to use Müller stem cells for efficient retinal neuron regeneration. Future studies will also need to address the central question of the neuronal fate control during the regeneration process, as it appears that the identity of the newly generated neurons poorly correlated to the identity of damaged neurons. Finally, it will be critical to determine how the combination of different transcription factors acting upstream or in parallel of Ascl1 will allow to increase cell proliferation and neuronal regeneration.

Conclusion

The replacement of lost retinal neurons is critical for many patients suffering from low vision or blindness. While stem cells transplantation is promising, this strategy comes with some important complications. Alternatively, cellular reprogramming of endogenous NSCs allows to bypass most of these issues. The characterization of the NSCs-dependent retinal regeneration has mainly been focused on injury-induced neuronal regeneration. While this strategy has increased considerably our knowledge in the molecular factors involved in this regenerative process, the current challenge is to control Müller cells derived neural progenitors proliferation and differenti-

ation without injury. Deciphering the molecular and cellular mechanisms controlling retinal NSC function in a powerful regenerative model as the zebrafish is an exciting starting point to identify future strategies fully exploiting mammalian Müller cells potential for human retina repair.

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Open peer review report:

Reviewer: Chih-Li Lin, Chung Shan Medical University, China.

Comments to authors: This is a perspective article for the topic of neuronal regeneration about retinal neural stem cells. The author highlighted the possible approaches of stem cells transplantation of retinal repair, and reviewed recent progress of endogenous neural stem cell reprogramming, particularly in the animal model of zebrafish. Overall, the novelty level was extremely high, and the major concept of current manuscript is quite interesting.

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